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THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

WOODLAND AVENUE AND THIRTY-SIXTH STREET, PHILADELPHIA 4, PA.

A STUDY OF THE DIET AND NUTRITIONAL STATUS OF WOMEN IN A LOW-INCOME POPULATION GROUP [1]

JET C. WINTERS AND RUTH E. LESLIE,
WITH THE TECHNICAL ASSISTANCE OF
MARGARET ANNE EPPRIGHT
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(Received for publication May 19, 1943)

The variation of vitamin and mineral content of food materials with such factors as source and season and the recognized but not readily assessed losses during storage, preservation, and preparation make a study of the nutritive value of diets as they are actually consumed of considerable interest. In this investigation a study was made of the diets of twenty-four women from the low-income group, all of whom lived, during the time they participated, in the various projects of the Austin Housing Authority. Anglo-Americans, Latin-Americans, and Negroes were represented. Diets were collected during one, two, or three weekly periods from November to June and examined quantitatively with respect to thiamine, riboflavin, niacin, pantothenic acid, protein, calcium, phosphorus, and calories. The analytical findings were correlated with data obtained by physical examination of fifteen of the subjects for anatomical evidences of deficiency.

EXPERIMENTAL

Selection of subjects

The subjects selected for study were residents of the three housing projects located in Austin, Texas and included Anglo-Americans, Latin-Americans, and Negroes. The mothers in

[1] This research was aided by a grant from the Williams-Waterman Fund.

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the families, chosen on the recommendation of the project director, were asked to participate because it was thought that they would be likely to be at home for all meals, would be interested, and would be more careful than any other member of the family in collecting the food samples. In order to have as homogeneous a group as possible, care was taken to select families of like size and income. The diets of five women from each racial group were collected for a period of 1 week at three different times during the year so that seasonal variation might be studied. From only eight of the women who cooperated in the study was it possible to obtain collections for all three periods as some of the families moved from the project, others had illnesses in the family, and some of the women decided that collecting the sample was too troublesome. Four women in both the Latin-American and Negro groups made collections for the three periods, but none of the Anglo-American women made collections for more than 2 weeks. The husbands in the families studied were occupied as plumber's helpers, day laborers, truck drivers, porters, etc. The weekly incomes of the twenty-four families ranged from ten to eighteen dollars and averaged thirteen dollars and fifty cents.

Collection of samples

The samples collected daily were, as nearly as possible, duplicates of the day's diet of the women participating, and included all meals and between meal snacks. Every effort was made to impress the women with the necessity of care in making their collections. They were instructed to have an extra plate on the table at each meal and to put on this plate exactly the same amount of food as they ate. At the end of each meal the food on the extra plate was to be transferred to a ½-gallon Mason jar, — milk, soup or other liquids consumed, except water, coffee, and tea, were to be put in a separate jar. The jars were furnished for this purpose. Corresponding portions of everything eaten between meals were also to be added to the jars. The women were asked to make lists of what they ate each day, so that the contents of the jar might be qualita-

tively checked. The women were reimbursed at the end of each weekly period in the amount of 25 cents per day.

The reliability of collections made in the way described evidently depends on the reliability of the subjects. In this study the cooperative spirit and interest of the women participating and the careful check made at the time of collection seemed to insure that the samples collected represented intake with reasonable accuracy. The probable reliability of the samples is discussed in more detail later on.

Preparation of samples

The solid food material was first ground in a food chopper. The liquid portion, together with water used in washing the grinder, was added and the mixture homogenized in a Waring Blendor. The homogeneous suspension was then made to a volume of 2 liters. For the dry weight determination a 20-ml. aliquot was dried in an electric oven at 70° C. for 24 hours. For thiamine assay, a 15 ml. aliquot was acidified with dilute H_2SO_4 (using Congo red paper) and the acidified solution steamed for 20 minutes. A portion of this acid extract was sulfite treated following the directions of Frey, Atkin, and Schultz ('42). For riboflavin, pantothenic acid, and niacin assay a 10-ml. aliquot was mixed with 30 ml. sodium acetate-acetic acid (0.5%) buffer, pH 4.5, 20 mg. each of clarase and papain added (approximately 2% by weight), and the solution placed in a 37° C. incubator for 24 hours. The sample was then steamed 30 minutes, cooled, restored to volume, centrifuged, tubed, and steamed 10 minutes for sterilization. Aliquots of this extract were used for the microbiological assays.

For the mineral assays 60 ml. of each of the seven samples for a period were combined, and a 25-ml. aliquot of this total sample digested by the perchloric acid method of Gerritz ('35) and eventually evaporated to between 20 and 50 ml. Complete hydrolysis was insured by the addition of 20 ml. water and further heating. The solution was then made to 250 ml. Aliquots of this 250-ml. solution were used for the mineral determinations.

After the sample necessary for the mineral analyses had been taken from the mixed week's sample, as described under preparation of sample for mineral assays, the remainder of the material was dried and used for protein assays and bomb calorimeter determinations.

Methods of analysis

1. The thiamine determinations were made with the fermentometer according to the procedure of Frey, Atkin, and Schultz ('42).

2. Microbiological methods were used for the determination of riboflavin, pantothenic acid, and niacin. These are fully described in The University of Texas monograph, "Studies on the Vitamin Content of Tissues," published under the direction of R. J. Williams ('41). The organisms used were obtained from the Biochemistry Department of The University of Texas.

3. The A.O.A.C. method ('35) was used for calcium assay. Phosphorus was determined colorimetrically by the method described by Koenig and Johnson ('42) using a Fisher electrophotometer.

4. Protein intakes were calculated from nitrogen values obtained by the usual macro-Kjeldahl procedure.

5. Bomb calorimeter determinations were made in the Chemistry Department of The University of Texas, under the supervision of Dr. John Griswold. Determinations were made on six samples from the Latin-American group and four each from the Anglo-American and Negro groups. All these samples with one exception were from the third collection period. The average figure of 5 calories per gram (dry weight) obtained from these analyses was used to calculate the calorie value of the food intakes.

Physical examination

Examination for anatomical evidence of vitamin deficiencies was made by Dr. Norman Jolliffe. The criteria used are summarized by Jolliffe and Stern ('42).

RESULTS

Table 1 presents for the twenty-four women participating the average daily intakes of calories, protein, calcium, phosphorus, and the four vitamins determined for the period or periods during which diets were obtained from the respective individual. The average daily intakes for each racial group and averages for all groups are shown. The vitamins are listed for which anatomical evidence of deficiency was discovered.

Riboflavin

Of all the women studied the average daily intake was 0.78 mg. per day, which is less than one-half the recommended daily allowance of 1.8 mg. for the sedentary woman. (Observation of the limited physical activity of the subjects led to their classification as sedentary.) In only one case (Subject B in the Negro group) did the intake equal the recommended allowance, but in no instance did it fall below 0.5 mg. (approximately one-fourth of the requirement). For nineteen out of the twenty-four women studied the average intake fell between one-fourth and one-half of the recommended allowance. This finding is emphasized by the incidence of cheilosis, corneal vascularity, or skin lesions in seven of the fifteen subjects examined by Dr. Jolliffe.

Pantothenic acid

The average intake of pantothenic acid was 2.41 mg. per day, with small variation for the three different groups. There is no "recommended allowance" for pantothenic acid, but an allowance of 10 mg. per day has been suggested by R. J. Williams ('42). The highest intake recorded in the present study was 4.15 mg., which again was for Subject B in the Negro group. A study of the data indicates that thirteen of the twenty-four subjects were receiving one-fourth to one-half the intake suggested as adequate.

TABLE 1

Average daily intakes and nutritional status of individual women

	NO. OF DAYS	ENERGY [1]	PROTEIN [2]	CALCIUM	PHOSPHORUS	RIBOFLAVIN	PANTOTHENIC ACID	NIACIN	THIAMINE	ANATOMICAL EVIDENCE OF DEFICIENCY IN [3]
		Cal.	*gm.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	
Anglo-American										
C	14	848	21.63	0.19	0.63	0.55	2.41	3.28	0.33	.....
L	14	1160	28.21	0.38	0.93	0.91	2.59	3.06	0.45	Riboflavin
G	14	1198	26.03*	0.27	0.72	0.52	2.36	4.22	0.43	.....
Ha	14	1610	31.08	0.45	1.22	0.74	3.06	5.70	0.75	Riboflavin, niacin, ascorbic acid
Hu	7	900	30.11	0.17	0.77	0.73	2.49	2.86	0.41	Vitamin A, niacin, ascorbic acid
S	7	1785	....	0.52	0.95	0.93	2.58	6.25	0.51	Vitamin A
W	7	915	....	0.40	0.38	0.95	2.79	5.25	0.47	.....
Ku	7	1465	45.36	0.40	0.90	0.83	2.85	7.65	0.66	None
T	7	1045	24.62	0.28	1.06	0.56	2.14	3.40	0.59	.....
De L	7	1435	45.66	0.48	1.98	0.88	3.58	4.01	0.56	Riboflavin, A, niacin, ascorbic acid
Kn	7	1370	41.43	0.51	1.37	0.81	3.11	4.41	0.60	Riboflavin, niacin
Average		1248	32.68	0.36	0.96	0.74	2.69	6.40	0.51	
Latin-American										
E	21	1230	31.15*	0.51	0.73	0.74	1.98	2.80	0.42	Niacin and ascorbic acid
F	21	863	26.93*	0.27	0.34	0.55	1.67	2.71	0.32	Riboflavin, niacin, ascorbic acid
G	21	1565	41.47*	0.55	0.55	0.72	2.55	2.78	0.59	.....
M	21	1235	35.08*	0.40	0.64	0.59	2.29	3.28	0.45	Ascorbic acid
Ra	7	1315	....	0.50	1.18	0.56	2.34	3.10	0.60	.....
Ram	7	1335	48.57	0.47	0.58	0.66	2.66	5.20	0.46	Vitamin A and ascorbic acid
S	7	1200	34.70	0.37	0.62	0.70	2.74	3.28	0.44	Riboflavin, niacin
Average		1249	36.32	0.44	0.61	0.65	2.21	3.09	0.45	
Negro										
B	21	1805	55.44*	0.65	0.82	1.80	4.15	9.76	0.92	Ascorbic acid, vitamin A
H	21	892	25.93*	0.25	0.56	0.62	1.77	3.21	0.42	Vitamin A, riboflavin, niacin
J	21	753	21.48*	0.28	0.27	0.62	1.62	3.80	0.27	.....
M	21	728	26.40*	0.27	0.37	0.75	1.72	3.27	0.35	.....
N	7	1620	46.59	0.60	0.73	1.02	2.69	5.86	0.82	Niacin, ascorbic acid
W	7	765	18.62	0.25	...	0.58	1.58	2.88	0.33	.....
Average		938	32.41	0.36	0.59	0.95	2.34	4.91	0.51	
Average for all groups		1145	33.80	0.38	0.72	0.78	2.41	4.13	0.49	

[1] Calculated on dry weights from an average value of 5 calories per gram obtained by bomb calorimeter determinations on representative samples.

[2] Starred values represent average of two periods of study; others one period.

[3] Examinations made by Dr. Norman Jolliffe on fifteen subjects.

Niacin

The average intake of niacin for all subjects was 4.13 mg. per day, with the intake of the Latin-American group definitely below the other two. Again Subject B in the Negro group had the highest intake found in this study, 9.76 mg. as compared to the recommended daily alowance of 12 mg. Three of the women had average intakes equal to more than one-half of the recommended allowance, and nineteen had average intakes above one-fourth of this amount. Nine of the fifteen subjects examined by Dr. Jolliffe showed the milder signs of niacin deficiency, such as edema and lateral redness of the tongue and red swollen papillae.

Thiamine

The recommended allowance for thiamine is 1.2 mg. per day. The average intake for the twenty-four women was 0.51 mg. per day. The highest daily intake recorded was 0.92 mg. for Subject B in the Negro group. Of the twenty-four average intakes presented eight are equal to one-half or more of the allowance and only five are less than one-third. Of the fifteen women examined by Dr. Jolliffe none showed diagnosable polyneuritis, but two showed plantar dysesthesia, which is the earliest objective evidence of polyneuritis.

While the diets studied were not assayed for vitamin A or ascorbic acid, the evidences for deficiency of these vitamins found by Dr. Jolliffe are included. Six subjects showed gross Bitot's spots, and all the remaining subjects had abnormal conjunctivae characteristic of xerosis. Nine women showed marginal gingivitis.

Calcium and phosphorus

A study of table 1 shows rather large variations in the calcium and phosphorus intakes of the individual women, ranging from 0.17 to 0.65 gm. for calcium and 0.23 to 1.98 gm. for phosphorus, with intakes of calcium and phosphorus well below recommended allowances. Eleven women had calcium

intakes less than one-half the recommended allowance of 0.8 gm. per day, and twelve had intakes from one-half to three-fourths the allowance. Two women had phosphorus intakes over the recommended allowance of 1.32 gm. Ten were below one-half the allowance. Nine of the remaining received one-half to three-fourths the allowance daily.

Protein

The average intake of protein for all the women studied was 33.80 gm. protein daily. Considerable variation in individual intake was encountered, the values ranging from 19 to 60 gm. The majority of the women apparently received from one-half to two-thirds the recommended protein allowance of 60 gm. per day. The average intake for each group and for all groups was below the amount estimated by Sherman ('41) as the minimum necessary for nitrogen balance in the adult man (44.4 gm.).

Calories

The caloric intake calculated as described above was surprisingly low for all groups. Individual intakes showed considerable variation, ranging from 620 to 2050 calories. The average for all the women was 1145 calories. When the intakes were compared with the standard allowance of 2100 calories for the sedentary woman, it was found that one-third of the women were consuming less than one-half the intake considered adequate, with half receiving from one-half to three-fourths the recommended allowance.

In order to indicate the extent to which the marked calorie deficiency might be correlated with the low protein, mineral, and vitamin values, the average intakes of these nutrients for each group were calculated to a 2100 calorie basis. These calculations indicated that a consumption of enough of the same kind of food as was eaten by the women cooperating in this study would have meant nearly adequate intakes of protein, phosphorus, and calcium and great improvement in vitamin intakes. If 2100 calories had been consumed the average

intakes of thiamine and riboflavin would have been approximately 75% adequate, niacin 62%, and pantothenic acid 43%.

Group differences in intake

On the whole the data show no great differences in the vitamin intakes of the three groups. The average intakes for the twenty-four women studied were slightly more than one-third the recommended allowance for thiamine, niacin, and riboflavin and one-fourth the suggested allowance for pantothenic acid. Phosphorus intakes more nearly approached the recommended allowance than did the calcium intakes, except in the Latin-American group. The calcium deficiency was more marked than the phosphorus deficiency in both the Anglo-American and Negro groups. The calcium intake for the Latin-American group was 25% greater than that of the other two groups, and the phosphorus intake was 50% greater in the Anglo-American than in the other groups. Very little difference was shown in the average daily protein intake of the three groups. The average caloric intakes for the Anglo-American and Latin-American groups were the same and were about 60% of the recommended allowance. The average intake for the Negro was 25% less than for the other groups and only 45% of the recommended allowance.

Seasonal variation

With the intention of discovering seasonal variation diets were collected from the Anglo-American women in November, February, and April, from the Latin-Americans in December, February–March, and May, and from the Negroes in January, March, and June. Since the make-up of the Anglo-American group varied from period to period, it was impossible to draw valid conclusions. Four of the women in both Latin-American and Negro groups made the collections for the three periods. In all three cases, however, averages for the periods show relatively small variation. This seems to indicate that seasonal availability of various products is a factor of little importance in the food selection of these low-income urban groups.

Physical findings

There is a fairly close correlation between the analytical data and the findings of physical examination except in the case of thiamine where the incidence of mild polyneuritis might have been expected. The absence of striking symptoms may be partially accounted for by the low caloric intake and perhaps by the adjustment of these people over a lifetime to diets uniformly deficient in calories, minerals, protein, and vitamins. Certain significant, although non-specific, symptoms were reported, twelve of the fifteen women examined stating that they were nervous or suffered with "nerves." Six experienced frequent headaches, and eight stated that they were easily fatigued.

DISCUSSION AND CONCLUSIONS

The method of computing intake of nutrients by analyzing a duplicate of the food consumed has not so far as is known, been used previous to the present investigation. The commonly used methods of ascertaining dietary intake of individuals are (1) analysis of aliquots of a weighed diet, and (2) computations from food-intake records. It was not possible to use the first of these in the present study and it was thought that the assay of duplicates possessed marked advantages over diet-record computations since it involves food as actually consumed and avoids the many errors inherent in the use of laboratory hand-book figures. Exact duplicates of intake cannot, of course, be obtained without weighing every portion, but if the collector is conscientious the errors involved over a week's period tend to cancel each other and the duplicate represents actual intake with reasonable accuracy. This was proved by collecting samples both with and without weighing. The chief source of error lies in lack of care in collecting the duplicate. The possibility that women in a low-income group would be tempted to collect smaller portions than were actually consumed suggests itself. Observation of and acquaintance with the women participating in this study indicate that small

samples were not due to "cheating" but to poor physical condition, lack of appetite and, especially, to unpalatable food. The samples were always called for by the investigators themselves and the women were questioned when the samples were particularly small. Minor illnesses and lack of appetite were the usual reasons given. Only in the case of milk was the economic factor mentioned. The mothers bought milk for the children, but could not afford it for themselves. The economic factor was, of course, indirectly involved since it determined, in general, the kind of food bought. Table 2 lists the foods consumed by the six women having the lowest calorie intake and makes evident the unattractiveness and monotony of the daily diet. Moreover, food preparation was almost invariably poor. Whether as a cause or result of the low-calorie intake, the restricted activity of the women is a factor worthy of comment. In general, their housekeeping standards were low and their outside activity negligible. Conversation with the women revealed the following picture of their activities and food habits:

> The mother gets up fairly early in the morning and prepares breakfast for the rest of the family. She does not eat her own breakfast until after the husband has gone to work and the children to school. By this time she is too tired to eat very much and often goes back to bed for additional rest. Lunch is only a snack since the husband and children are away and no regular meal is prepared. At night, after preparing dinner for the family, the mother is again too tired to eat normally.

When the first assays were completed and the low figures became apparent, efforts were redoubled to make sure that complete samples were obtained. The women were encouraged to report any item for which a duplicate had not been available, e.g., an egg, glass of milk, slice of bread, etc. so that it might be secured and added to the diet.

In comparison with the recommended allowances the intakes reported are unusually low and the correlation between intakes and physical condition is not as high as might be expected. The intakes are also lower than are reported in other studies of low-income groups. However, most of these other studies have been done on the family group as a whole and results re-

TABLE 2

Typical daily food lists of low-income women [1]

Subject M — Negro

1. Cornmeal mush, prunes, string beans, potatoes, white bread.
2. Cabbage, hamburger, white bread.
3. Potatoes, carrots, roll.
4. Cornmeal mush, string beans, carrots, cauliflower, cornbread, toast.
5. Rice, ham, white bread, kidney beans, vegetable salad, cornbread, cake.
6. Rolls, fresh peas, macaroni, ham, omelet.
7. Rice, white bread, cabbage, fresh peas, hamburger, cornbread.

Subject J —Negro

1. Grits, lettuce, potatoes, rolls, gravy.
2. Lima beans, white bread, grits, prunes.
3. Cornbread, weiners, applesauce, macaroni and cheese.
4. Dried beans, sweet potatoes, cornbread, grits, white bread.
5. Spinach, potatoes, hamburger, cornbread, grits, white bread.
6. Greens, cornbread, potatoes, oatmeal, white bread.
7. Potatoes, lettuce, bacon, white bread, oatmeal, milk, white bread.

Subject H — Negro

1. Cabbage, meat, potatoes, cake, peas, white bread, tomatoes, cereal, prunes, milk.
2. Beans, prunes, white bread, crackers, spinach, potatoes, grits.
3. Cabbage, peas, potatoes, salmon, white bread, crackers, cake, cereal.
4. Potatoes, tomatoes, white bread, peanut butter, crackers, cereal.
5. Soup, potatoes, crackers, white bread, cereal, milk.
6. Beans, potatoes, sardines, white bread, cereal.
7. Cabbage, salmon, potatoes, white bread, peanut butter, cereal.

Subject F — Latin-American

1. Cream of wheat, beans, cheese, onions, white bread, jelly, chili con carne.
2. Bacon, egg, enchilada, meat, potatoes, cocoa.
3. Oatmeal, cookie, white bread, corn, lemon pie, beans, gravy, milk.
4. Oatmeal, toast, enchilada, beans, crackers, peaches, spinach, eggs, white bread, milk.
5. Egg, toast, potatoes, white bread, peanut butter, orange juice, pork and beans, green peppers, egg, white flour tortillas.
6. Egg, toast, cocoa, bread, crackers, potatoes, hamburger, tortillas, beans.
7. Egg, bread, gravy, milk, beans, crackers, mayonnaise.

Subject Hu — White

1. Potatoes, bacon, biscuit, raisins, cornbread, chili, crackers, beans, oatmeal.
2. Cornbread, vegetable soup, white bread, beans, raisins, oatmeal, biscuits.
3. Sausage, white bread, gravy, lettuce, bacon, fresh peas, fresh peaches, honey, oatmeal.
4. Potatoes, peas, gravy, bacon, bread, hot cakes, syrup, biscuit, oatmeal.
5. Dried beans, cornbread, potatoes, sweet potatoes, dried peas, boiled pork, lettuce, white bread, oatmeal, milk.
6. Potatoes, bacon, white bread, chocolate custard, sweet potatoes, soup.
7. Egg, apple, white bread, bacon.

Subject C — White

1. Potatoes, macaroni, pickles, white bread, jello.
2. Dried beans, vegetable soup, crackers, stewed apple.
3. Spaghetti, crackers, egg, toast.
4. Dried beans, potatoes, bread pudding, okra and tomatoes, crackers, white bread.
5. Potatoes, okra and tomatoes, bread, peanut butter, crackers.
6. Potatoes, white bread, liver, gravy.
7. Potatoes, crackers, tomatoes.

[1] Coffee, sugar, and butter or oleomargarine also appear on the daily diets, but are not recorded.

ported on a "unit" basis. Hunter and Pett ('41) in a dietary survey of 76 families in which intakes for each member of the family were separately calculated concluded that food was not fairly distributed in the family and that the worst fed member was the mother. They state that 78% of the women were deficient in calories and only 42% of the men. Similar figures for calcium are 66% in comparison with 28%, for iron 95% with 5% and vitamin A 80% with 52%. Actual figures on intake are not given, except the average for the group as a whole, but it was shown that more of the families on a yearly income of $500–$1000 were deficient than of the families on incomes of $1000–$1500. Families in the study here reported were within the $500–$600 income range.

Milam ('42) in a dietary survey of a small North Carolina community, stated that the average calorie intake of that portion of the community over 15 years of age was 2000. This corresponded closely with the intake of a group of doctors and technicians at Duke Hospital. For only part of his study does he give separate calorie intakes for women and these are stated to be 1577 for whites and 1443 for Negroes.

Youmans et al. ('43) in a study of a rural population in Tennessee obtained calorie intake figures that are somewhat more comparable to those reported here. Calories were calculated from a 3-day intake record. The group studied comprised 776 whites and 385 colored. Included in this group were 229 adult white and 96 adult colored females. Of this total group of 325, 29.1% had calorie intakes below 1250; in the white group the percentage was 18.6% and in the colored 54.9%. Youmans points out that the recorded calorie intakes are not only relatively low but absolutely so low that their continuance for any but the shortest periods would seem inevitably to be followed by disabling loss of weight and strength. Weight records, however, showed that only 5% of the colored women were underweight and 10% of the white. In other words a larger percentage of the colored than the white women had calorie intakes below 1250, but a much smaller percentage were underweight. A more intensive study was made on a representative

10% of the group and it was shown that as many as one third of the group had intakes less than 30% above actual basal requirements while several had intakes that were below. Moreover, only a small part of this group were as much as 20% below their predicted "ideal" weight and many were over that weight by as much as 30%.

Very few of the group represented in the present investigation were underweight and several were considerably overweight. Calorie intake figures obtained in the present investigation tend to confirm the lack of correlation noted by Youmans between estimated calorie intake and body weight. Results obtained by the present investigators in a further study (to be presented later) in which diet samples were collected from a higher income group and from a group in which the collection of samples was done with special care, are similar to those obtained in the present study. Moreover, the large number of caloric intakes below 1250 found by Youmans is in accord with the findings of the present study of an average of 1248 for the white, 1249 for the Latin-American, and 938 for the Negroes. Four of the six Negro women studied had exceptionally low intakes. Youman's figures on protein, mineral, and vitamin intake have not yet appeared, but it is probable that low-calorie intakes will be accompanied by low intakes of other factors. Youmans raises the question of whether the recommended allowances have been placed too high, and it would seem that enough data have accumulated to make this possibility worthy of further investigation.

SUMMARY

The study of three hundred eight separate diets collected from twenty-four women of the low-income group for weekly periods at three different seasons revealed severe deficiencies in calories, protein, minerals, and vitamins. When the average daily intakes were compared with the daily allowances for the sedentary woman recommended by the Committee on Food and Nutrition of the National Research Council, it was found that the calorie intake was from one-half to three-fourths the al-

lowance, the average intakes of thiamine, niacin, and riboflavin, slightly more than one-third, and protein, calcium, and phosphorus approximately one-half the amounts recommended as adequate. The pantothenic acid intake was about one-fourth the amount suggested by R. J. Williams ('42) as representing adequacy for this vitamin. Seasonal variation and differences between the intakes of Anglo-American, Latin-American, and Negro subjects were found to be slight.

The findings of physical examination of fifteen of the twenty-four women corroborate the data obtained from the dietary assay for the B-complex of vitamins. The fact that no extreme cases of deficiency were observed and that all cases did not show the same degree of deficiency on similar intakes does not alter the conclusion that among people of the low-income group represented in this study chronic nutritional deficiency is prevalent. On the other hand the absence of grave manifestations of malnutrition on the low intakes found, and particularly the failure to observe underweight as a result of the low calorie intakes, suggests the possibility that the recommended allowances have been placed too high. Reference is made to other investigations showing similar results.

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RIBOFLAVIN AND THIAMINE INTERRELATIONSHIPS IN RATS AND IN MAN[1]

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ONE FIGURE

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INTRODUCTION

Rats markedly deficient in thiamine have been found to deviate somewhat from normal in their metabolism of riboflavin. Supplee et al. ('42) observed that after the ingestion of food there is a less than usual increase in liver riboflavin in thiamine deficient animals. Sure and Ford ('42) found that the concentration of riboflavin in the tissues of rats suffering from severe thiamine deficiency was 10 to 20% less than that of their control animals. The suggestion of the latter authors that thiamine deficiency, by affecting riboflavin retention and utilization, may be of clinical significance in the production of riboflavin deficiency in man, has occasioned the present review of data which had been collected in the course of another investigation (Ferrebee, Weissman, Parker and Owen, '42).

METHODS

The details of the experimental procedures and the methods of analysis have been described elsewhere (Ferrebee, Weissman, Parker and Owen, '42; Ferrebee, '40 a, b; Ferrebee and Carden, '40). Male white rats of the Wistar

[1] Aided by a grant from the Williams and Waterman Fund, Research Corporation, New York City.

strain, weighing 180 gm., were placed on a thiamine deficient diet (Ferrebee, Weissman, Parker and Owen, '42). The concentrations of riboflavin and thiamine in their livers and kidneys were compared at intervals of 1 to 4 weeks with the concentrations observed in control animals which received the thiamine deficient diet with either unautoclaved yeast or thiamine.

Measurements were made of riboflavin and thiamine excretion in the control and in the deficient animals, both during fasting and after the administration by various routes of known amounts of riboflavin and thiamine. All animals were fasted overnight before they were killed for tissue analyses or used for excretion tests. During the excretion tests animals were fasted, but allowed water ad libitum.

In a group of patients of varying nutritional status, measurements were made of tissue riboflavin and thiamine concentrations and urinary thiamine and riboflavin excretion. The excretion experiments were performed in the morning on fasting patients who had received 200 to 400 ml. of water ½ hour before the injection of vitamin. The measurements of vitamin excretion were accompanied by measurements of blood urea and of urinary urea excretion.

RESULTS

A. Experiments on rats

Figure 1 illustrates the effect of a thiamine deficient diet upon the growth curve of 180 gm. male Wistar rats (Ferrebee, Weissman, Parker and Owen, '42). Isocaloric feeding does not maintain parallelism in growth in the control and thiamine deficient animals (Sure and Dichek, '41; Sure and Ford, '42). No effort was made to secure equal caloric intakes in our control and thiamine deficient groups. During the third and fourth week of the experiment the deficient animals lost weight and their livers were found to be small and the capsule shrunken and white.

Table 1 presents data on the concentration of riboflavin and thiamine in the livers and kidneys of a group of four-

teen control and fourteen thiamine deficient animals. Thiamine concentrations fell to critical levels in about 2 weeks. Weight loss begins at this time and apparently delays further fall in thiamine concentrations (Ferrebee, Weissman, Parker and Owen, '42).

In general the concentration of riboflavin in the livers of our thiamine deficient animals is lower than that observed in the control group. However, judging from the variations observed in both groups, this difference is not of great phy-

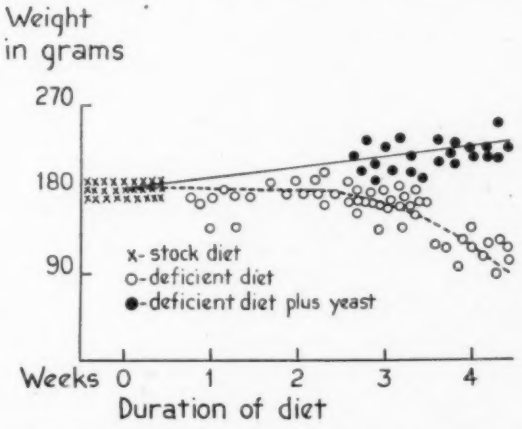

Fig. 1 Effect of thiamine deficient diet on growth curve of 180 gm. male Wistar rats.

siological or clinical importance. Furthermore, it is evident that progressive thiamine deficiency does not cause a progressive fall in tissue riboflavin concentrations. The liver riboflavin concentrations at the end of 4 weeks of thiamine deficiency are essentially equal to those observed in the earlier periods of deficiency. The differences in kidney riboflavin concentrations in the control and in the thiamine deficient groups throughout the experiment appear scarcely worthy of comment.

Table 2 presents data on the excretion of riboflavin and thiamine in eleven control animals and in eleven animals

that had been on a thiamine deficient diet for a period of 2 weeks. It may be seen that the individual variations in the excretion of riboflavin exceed the differences which may be detected between the control and the thiamine deficient group. For comparison, data are also given from a num-

TABLE 1

Riboflavin and thiamine concentrations in livers and kidneys of thiamine deficient rats.

(Concentrations expressed in micrograms per gram of fresh tissue.)

DURATION OF DIET	RIBOFLAVIN IN LIVER		RIBOFLAVIN IN KIDNEY		THIAMINE IN LIVER		THIAMINE IN KIDNEY	
	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient
3 days	20	23	20	23	9	4	4	3
	20	29	21	27	10	6	5	3
	20	27	19	25	10	5	5	3
8 days	31	21	24	26	12	2	6	1
	36	26	20	28	9	2	5	1
	28	23	25	24	11	2	6	1
2 weeks	21	27	21	24	8	1	5	1
	30	26	21	25	9	1	4	1
3 weeks	33	24	31	24	11	1	5	1
	23	24	32	24	8	1	3	1
	35	22	33	22	9	1	6	2
	40	24	27	27	8	1	5	1
4 weeks	35	20	29	25	10	1	5	1
	36	26	39	25	11	1	7	1
Average of 3 and 4 week periods.	34	23	32	25	10	1	5	1

ber of tolerance tests for riboflavin and thiamine excretion performed on a similar group of animals after subsisting for 2 weeks on a thiamine deficient diet. The data of table 2 give little indication that thiamine deficiency of 2 weeks' duration affects riboflavin excretion.

TABLE 2

Urinary excretion of riboflavin and thiamine by rats which have been on a thiamine-deficient diet for 2 weeks: Basal excretion, and excretion after small test quantities of riboflavin and thiamine.

DURATION OF URINE COLLECTION	RIBOFLAVIN EXCRETED (MICROGRAMS)		THIAMINE EXCRETED (MICROGRAMS)		TYPE OF EXCRETION TEST
	Control	Deficient	Control	Deficient	
24 hrs.	66	28	4	1	
	51	49	3	1	Basal excretion: thiamine deficient diet for 2 weeks.
	42	26	3	1	
	34	38	2	1	
Average	43	35	3	1	
3½ hrs.	4	10	0.6	0.3	
	4	7	0.6	0.3	Basal excretion: thiamine deficient diet for 2 weeks.
	9	9	0.7	0.3	
	8	8	0.9	0.3	
Average	7	9	0.7	0.3	
1½ hrs.	3	1	0.3	0.1	
	5	3	0.6	0.2	Basal excretion: thiamine deficient diet for 2 weeks.
	4	3	0.3	0.2	
Average	4	2	0.4	0.2	
24 hrs.	46	36	10	2	
	48	40	9	1	160 μg. riboflavin and thiamine per os in 4 ml. normal saline.
	48	65	7	8	
	42	25	6	2	
Average	46	42	8	3	
3½ hrs.	21	27	7	0.5	
	43	23	16	0.4	40 μg. riboflavin and thiamine injected subcutaneously in 1 ml. normal saline.
	20	22	6	0.5	
	..	22	..	0.5	
Average	28	23	10	0.5	
1½ hrs.	5	20	5	2	40 μg. riboflavin and thiamine injected intravenously in 1 ml. normal saline.
	15	20	9	3	
	15	21	13	5	
Average	12	20	9	3	

B. Experiments on man

Table 3 presents data on the concentrations of riboflavin and of thiamine found in the tissues of presumably "normal" individuals, compared with the concentrations found in the tissues of individuals with "poor" nutrition. The clinical material is not sufficient to permit generalizations, but one or two comments may be pertinent.

TABLE 3

Concentrations of riboflavin and thiamine in human tissues. (Micrograms per gram of fresh tissue.)

SUBJECT	AGE IN YEARS	SEX		HEART	SKELETAL MUSCLE	LIVER	KIDNEY CORTEX	CEREBRAL CORTEX	REMARKS
Presumably "normal" individuals.	10	M	Riboflavin	8.7	4.0	15.7	16.1	..	Food in stomach, traumatic death
			Thiamine	3.5	1.2	1.4	2.1	..	
	30	M	Riboflavin	6.4	1.6	14.1	11.8	2.2	Negro, traumatic death
			Thiamine	2.4	0.4	1.0	1.8	1.1	
	52	M	Riboflavin	5.4	1.2	9.9	10.1	1.6	Traumatic death, survived 24 hours
			Thiamine	2.0	0.4	1.2	1.3	0.8	
Patients with "poor" nutrition.	34	F	Riboflavin	6.9	1.2	8.3	9.1	..	High cord injury, sepsis 3½ months
			Thiamine	0.9	0.2	0.7	1.0	..	
	38	F	Riboflavin	4.8	1.3	3.2	4.8	1.1	Active tuberculosis of spine and adrenals, high fever
			Thiamine	0.6	0.0	0.3	0.4	0.5	
	49	F	Riboflavin	6.4	1.5	11.6	3.6[1]	..	Lymphosarcoma, uremia; high fever for 3 weeks
			Thiamine	0.5	0.1	0.6	0.3[1]	..	

[1] Kidney infiltrated with tumor.

The tissue analyses (table 3), show that only one of the patients with "poor" nutrition was deficient in riboflavin. This was the patient with tuberculosis of the spine and adrenals, who had been anorexic for some weeks and had been maintained on glucose and saline infusions during a terminal febrile illness of 2 weeks' duration. In this individual the concentrations of riboflavin in the liver and in the kid-

neys were considerably less than normal. The concentrations of riboflavin in the heart and in the skeletal muscles, on the other hand, were essentially normal for her age group. The relatively normal concentration of riboflavin in the muscles of patients thought to have riboflavin deficiency has received previous comment (Axelrod, Spies and Elvehjem, '41).

The data on both riboflavin and thiamine concentrations (table 3) reveal that the two other individuals with "poor" nutrition suffered from thiamine deficiency but not from riboflavin deficiency. In other words, their riboflavin nutrition was not affected by a rather considerable degree of thiamine deficiency.

In a group of fourteen determinations of the simultaneous excretion of riboflavin and thiamine, it was found in eight normal individuals that following intravenous injection of 1 mg. of each of the vitamins, 20 to 25% of the thiamine injected, and 30 to 35% of the riboflavin, appeared in the urine within 1 hour (Ferrebee and Carden, unpublished data). The values for the 2-, 3-, and 24-hour excretions following this type of test were in agreement with the observations reported by Najjar and Holt ('41) and will not be given in detail.

Table 4 presents data obtained when the 1-hour intravenous test was applied to a group of four patients suspected of dietary deficiency. Blood urea concentrations in this group were normal (Najjar and Holt, '40).

It may be observed that essentially normal values for both riboflavin and thiamine excretion were obtained in patients "Bz" and "Cy" and that a greater than usual excretion of riboflavin and thiamine was observed after several days of vitamin therapy.

Excretion tests indicated that thiamine deficiency was present in the two other patients, "Ns" and "Br," and this thiamine deficiency again did not seem to be accompanied by evidences of riboflavin deficiency (Najjar and Holt, '41).

The excretion of thiamine by patient "Ns" was less than normal on two occasions and the abnormality was not associated with abnormality of general renal function, that is, urea, water, or riboflavin excretion. The excretion of urea

TABLE 4

Per cent of administered vitamin excreted in 1 hour following intravenous injection of 1 mg. of riboflavin and 1 mg. of thiamine in four subjects suspected of dietary deficiency.

PATIENT	DATE	STATUS	MILLILITERS OF URINE EXCRETED	GRAMS OF UREA EXCRETED	PER CENT OF RIBOFLAVIN TEST EXCRETED	PER CENT OF THIAMINE TEST EXCRETED
Bz	6-15-40	Anorexia nervosa	295	..	26	21
	6-17	Anorexia nervosa	282	.62	26	19
	6-24	10 mg. B_1 and B_2 for 5 days	537	1.10	44	33
Cy	6-15-40	Alcoholic polyneuritis	110	..	24	21
	6-17	Alcoholic polyneuritis	51 [1]	.43 [1]	17 [1]	15 [1]
	6-24	Yeast tablets daily for 1 wk.	325	1.50	52	30
Ns	6-15-40	Diet low in B complex	175	..	25	13
	6-17	Diet low in B complex	170	.65	23	12
	6-24	10 mg. B_1 and B_2 for 5 days	105	.68	39	27
Br	7-2-40	Congestive heart failure, ? thiamine deficiency	250	1.70	46	9

[1] Specimens probably incomplete.

and water was unchanged by vitamin therapy; but the thiamine excretion returned to normal, indicating that the previous low values for thiamine excretion were related to the suspected thiamine deficiency. The excretion of thia-

mine by patient "Br" was considerably less than normal, the values for water, urea, and riboflavin excretion being at the same time elevated by a moderate diuresis caused by bed rest and cardiac therapy.

DISCUSSION

The observations reported fail to demonstrate that thiamine deficiency per se has an effect upon riboflavin metabolism. Animals maintained from infancy on markedly deficient diets (Supplee, Jensen, Bender and Kahlenberg, '42) or maintained in thiamine deficiency to the point of death (Sure and Ford, '42) may show some slight deviations from normal in their tissue riboflavin concentrations or in their urinary riboflavin excretion. These deviations are by no means clinically impressive and their occurrence is not particularly surprising in view of the extreme degree of secondary malnutrition and general marasmus exhibited by the animals in question.

The observation that thiamine deficient animals in the later stages of deficiency lose weight rapidly is ample explanation for the increased excretion of riboflavin observed in that period (Sure and Ford, '42). Our observations indicate that an increased excretion of riboflavin is not observed in the earlier stages of thiamine deficiency before weight loss is prominent, despite the fact that tissue thiamine concentrations at this time have reached levels comparable to those maintained in the later stages of deficiency (Ferrebee, Weissman, Parker and Owen, '42). The increased excretion of riboflavin appears therefore to be an unspecific phenomenon of the late stages of thiamine deficiency related to the rapid metabolism of tissues that occurs when the thiamine requirements can be satisfied only by tissue loss. The shrinkage of the liver observed in this period is sufficient in itself to explain the origin of a great part of the riboflavin that appears in the urine at this time. Excretion of this riboflavin, representing as it does an excretion of some of the unused products of tissue catabolism

(Sure and Dichek, '41) can scarcely be taken as evidence of riboflavin deficiency. In fact, it might be argued that in so far as tissue catabolism makes riboflavin, as well as thiamine, available from tissue sources, the dietary requirements for riboflavin may be reduced during periods of severe thiamine deficiency. The common observation that evidences of multiple or secondary deficiencies frequently make their appearance during the curative phase of primary deficiencies, rather than during the acute phase of these deficiencies, is corroborative of this view. This suggests as a corollary that the dietary requirements for riboflavin and other vitamins may be increased during the period of weight gain and tissue reconstitution which follows thiamine therapy. Vitamin interrelationships of this type have been illustrated and discussed by Morgan ('41).

As for the suggestion that thiamine deficiency may be of significance in the production of riboflavin deficiency in man, it may be observed that the above arguments are applicable to this question and that clinical investigation has failed to disclose an obligatory association of the two deficiencies. In the writers' experience it has been difficult to obtain objective evidence that thiamine deficiency, either clinical or subclinical, is of frequent occurrence in the population we have studied. Tissue analyses and excretion experiments in those few instances in which thiamine deficiency has been demonstrable have not shown that deficiency of this vitamin has an effect upon riboflavin metabolism. In patients whose vitamin nutrition is generally "poor," the necessity for distinguishing riboflavin deficiency due to inadequate riboflavin intake from riboflavin deficiency due to a presumed relationship to thiamine deficiency is at once apparent. Thus it would seem reasonable at the moment to consider that our one patient with evidences of both riboflavin and thiamine deficiency (the patient with tuberculosis of adrenals—table 3), suffered an inadequate intake of both riboflavin and thiamine during the period of her maintenance on glucose infusions. In our four other patients with thia-

mine deficiency neither tissue analyses nor measurements of riboflavin excretion gave evidence that thiamine deficiency had affected their metabolism of riboflavin.

CONCLUSION

Thiamine deficiency is not of clinical significance in the production of riboflavin deficiency. Changes in riboflavin metabolism are observed only in the terminal stages of thiamine deficiency and appear to be unspecific and of minor importance.

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SOME OBSERVATIONS ON ABRINE[1]

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The substance, abrine, was isolated by the Indian workers, Ghatak and Kaul ('32), in the course of a chemical study of the seeds of Abrus precatorius, a plant of the natural order Leguminosae. The seeds (known as jequirity seeds) are common in tropical countries and have been an article of Hindu materia medica from a very remote period. Abrine was found to occur free in the seeds from which it could be extracted by appropriate solvents. The investigators in India described certain tests for the crystalline material which they obtained, but it remained for the Japanese investigator, Hoshino ('35), to reveal that abrine was chemically related to tryptophane. Cahill and Jackson ('38) have shown that this naturally occurring N-methyltryptophane belongs to the same configurational series as *l*(—)-tryptophane.

We thought it of interest to determine whether or not the methyl group of abrine, like the methyl group of methionine, was labile in the sense that it might be readily transferable in vivo and thus render abrine lipotropic under certain conditions. In a second experiment we sought to determine whether abrine could prevent the development of cataract in rats maintained for a prolonged period on a tryptophane-deficient diet.

[1] This study was supported by a grant from Frederick Stearns and Company, Detroit, Michigan.

These experiments were reported at the meeting of the Michigan Academy of Science, Arts and Letters at Ann Arbor on March 26, 1943.

EXPERIMENTAL

Abrine for the present study was prepared by a procedure involving extraction of the ground seeds of Abrus precatorius with methanol, as described in detail by Cahill and Jackson ('38). It should be pointed out that a toxic protein, abrin, occurs in the seeds and a mask should be worn while they are being ground. With regard to the toxicity of abrin it has been stated that the "smallest particle in slightest wound may prove fatal." (Merck Index, '40.) Owing to similarity in spelling, abrine is unfortunately sometimes confused with abrin in the literature. The substance, abrine, has no apparent untoward effect on rats to which it is fed.

One sample of abrine employed possessed a specific rotation of $+45.3°$ at 25°C. in 0.5 N HCl, another a rotation of $+45.7°$. Values of $+46.0°$ (Cahill and Jackson, '38) and $+44.4°$ (Hoshino, '35) have been reported.

Fatty liver experiments

In the study of the possible lipotropic action of abrine, the experimental procedure described by Tucker and Eckstein ('37) in their investigation on the lipotropic activity of methionine was followed. Male white rats of the Sprague-Dawley strain, weighing approximately 120 to 145 gm., were fed the diets shown in table 1 for 19 days, at the end

TABLE 1

Diets employed in fatty liver experiments.[1]

DIET	I	II	III	IV
	%	%	%	%
Casein	5.0	5.0	5.0	20.0
Glucose	48.0	47.5	47.3	33.0
Agar	2.0	2.0	2.0	2.0
Lard	40.0	40.0	40.0	40.0
Salts (Wesson)	5.0	5.0	5.0	5.0
dl-Methionine	...	0.5	...	...
l(+)-Abrine	...	...	0.73	...

[1] The diet of each experimental animal was supplemented daily with 400 mg. of dried yeast and 2 drops of cod liver oil.

of which time they were decapitated and the total fatty acids in the liver determined essentially according to the method described by Leathes and Raper ('25). The results of this study are shown in table 2. As can be seen in table 2, the livers of the animals fed a 20% casein diet, or a 5% casein diet supplemented with methionine, contained much less fat than the livers of the animals receiving abrine. The amount of abrine fed in diet III was comparable on a molar basis with the supplement of methionine in diet II. The fatty acid content of the livers of the animals receiving abrine in the diet was similar to the fatty acid content of the livers of the unsupplemented controls. The results indicate that abrine is not significantly lipotropic.

TABLE 2

Results of fatty liver experiments.

DIET	NUMBER OF RATS USED	AVERAGE DAILY FOOD CONSUMPTION	AVERAGE WEIGHT OF LIVER	$\frac{\text{LIVER WT.}}{\text{BODY WT.}} \times 100$	TOTAL FATTY ACIDS IN LIVER: AVERAGE AND RANGE
		gm.	*gm.*		%
I	10	6.49	5.87	4.43	24.2 (10.2–38.2)
II	5	6.25	6.15	4.39	14.8 (9.0–22.7)
III	10	6.47	5.61	4.30	22.2 (13.9–34.1)
IV	4	7.03	6.39	3.80	15.6 (12.7–18.2)

It is possible that in the metabolism of N-methyltryptophane the first step may consist in the removal of methyl amine rather than the methyl group alone. Information on this point was sought by incubating abrine, dissolved in a modified physiological salt solution (Krebs, '33), under an atmosphere of oxygen with kidney slices. Abrine belongs to the *l*-configurational series and for this reason human kidney slices were employed after rat kidney slices yielded no positive

result. In this connection, it has been shown that a human kidney slice will deaminate a naturally occurring amino acid as rapidly as it does the unnatural optical isomer, whereas slices of the kidneys of rats and a number of other animals deaminate the *l*-series amino acids at only a fraction of the rate at which they attack amino acids of the *d*-configurational series (Krebs, '33). Tests for methyl amine after incubation even with human kidney slices were, however, inconclusive.

Cataract experiments

It has been shown by Totter and Day ('42) that the inclusion of as little as 0.1% tryptophane in the diets of animals not receiving this essential amino acid will prevent the development of cataract which is observed in the eyes of rats fed a tryptophane-deficient diet for prolonged periods. Excellent descriptions of the cataract have been reported by Totter and Day ('42) and by Albanese and Buschke ('42). In our study three groups of weanling white rats were fed the acid-hydrolyzed casein diet described by Totter and Day ('42). The basal diet was made up of 14.7% acid-hydrolyzed casein,[2] 0.3% cystine, 15.0% sucrose, 42.0% starch, 2.0% agar, 2.0% salt mixture (Wesson, '32), 5.0% cod liver oil, 19.0% Crisco, and B vitamins were furnished by daily supplementation with 250 mg. of yeast.[3] One group received, in addition, 0.1% tryptophane in the diet, another group 0.107% abrine, and a third group served as a control. The duration of the experiment was 90 days. A summary of the results is shown in table 3.

Although the number of animals employed was small, owing to the limited supply of abrine available for these experiments, the results show clearly that *l*(+)-abrine, like *l*(−)-tryptophane, is able to prevent the occurrence of cataract in rats fed a tryptophane-deficient diet. Inclusion of abrine in the diet of tryptophane-deficient rats promoted

[2] We wish to thank Dr. Melville Sahyun of Frederick Stearns and Company for a gift of acid-hydrolyzed casein.

[3] Harris.

their growth. As shown in table 3, however, the growth resulting from supplementation with abrine was not equivalent to that induced by supplementation with a comparable amount of tryptophane. This is in agreement with Gordon ('39) who also observed that *l*(+)-abrine is not as efficient as *l*(—)-tryptophane in the promotion of growth when fed to rats deficient in tryptophane. This investigator has suggested that the conversion of abrine to tryptophane, or to some physiologically available precursor of this amino acid, is not quantitative.

TABLE 3

Results of cataract experiments.

DIET	NUMBER OF RATS USED	AVERAGE WEIGHT AT BEGINNING	AVERAGE WEIGHT AFTER 90 DAYS	NUMBER OF RATS WITH CATARACT AFTER 90 DAYS
		gm.	*gm.*	
Tryptophane-deficient	6	34.1	30.8 [1]	4 [1]
Tryptophane-deficient plus 0.1% *l*(—)-tryptophane	6	36.0	107	0
Tryptophane-deficient plus 0.107% *l*(+)-abrine	6	36.1	93	0

[1] 4 surviving rats.

SUMMARY

The naturally occurring N-methyltryptophane, *l*(+)-abrine, did not prevent the accumulation of fat in the livers of animals fed a diet deficient in the labile methyl group.

The growth promoting effect of abrine fed to rats on a tryptophane-deficient diet was confirmed.

The development of cataract in rats maintained on a ration deficient in tryptophane was prevented when the diet was supplemented with abrine.

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LOSSES OF B VITAMINS DUE TO COOKING OF FOODS [1]

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Much information has been obtained recently regarding the distribution of the various B vitamins in natural foodstuffs (Waisman and Elvehjem, '41; Lane, Johnson and Williams, '42; Cheldelin and Williams, '42). However, information is less complete regarding the amounts of these vitamins present in foods as they are actually eaten.

In a previous paper (Lane, Johnson and Williams, '42) the losses in thiamine due to cooking were determined for a large number of foods comprising the average American diet. The present investigation deals with similar losses in six other vitamins: riboflavin, nicotinic acid, pantothenic acid, biotin, inositol and folic acid.

EXPERIMENTAL

Selection and sampling of foods

Vegetables, fruits, milk and eggs were purchased in nearby markets in as fresh condition as possible. Meats were, with few exceptions, obtained from a single wholesale butcher so that it was possible in most cases to be assured of freshness of the samples. Fish were bought as frozen commercial products.[3] Perishable foods were kept at 0–5° C. until they were

[1] Presented before the Division of Biological Chemistry at the 104th meeting of the American Chemical Society, Buffalo, New York.

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[3] Birds-Eye.

prepared for assay. These preparations were made within a few hours after procurement of the samples.

Procedure

The microbiological assay methods developed in the University of Texas Laboratories (Williams, '41) have been employed throughout for each of the six vitamins herein discussed.

All food extracts were prepared by digestion with takadiastase and papain according to the general method described previously (Cheldelin, Eppright, Snell and Guirard, '42). This procedure has proved to be quite satisfactory for preparation of extracts of natural materials, although it has not been confirmed generally for use with cooked foods. It is possible that in certain cases vitamins which have become "bound" by cooking and thus rendered unavailable to microorganisms (or to the reagents used in various chemical tests) may still be available for animal nutrition. This is apparently true for thiamine in some tissues (Lane, Johnson and Williams, '42). Satisfactory animal assays are not available for several vitamins, however, and in the absence of further direct evidence we have chosen to employ the digestion procedure mentioned above. Vitamin losses which appear to be very large may warrant reexamination at a later time.

RESULTS

Tables 1 and 2 contain the details of cooking and the results of the analyses of samples of thirty foods before and after cooking for the six vitamins mentioned above.

Lane, Johnson and Williams ('42) have pointed out the difficulties in estimating the amounts of cooking waters to be used or discarded in the preparation of foods. In an effort to standardize these factors we have included cooking waters and meat juices as part of the cooked samples except in a few cases where the cooking waters were assayed separately and discarded. The values in table 2 therefore tend to represent maximum amounts of the various vitamins present after cooking.

TABLE 1

Details of the cooking of various foods.

FOOD	MANNER OF COOKING	TIME OF COOKING	WATER OR FAT ADDED	WEIGHT BEFORE COOKING	EDIBLE AFTER COOKING
Meats		*Minutes*	*gm.*	*gm.*	*gm.*
1.[1] Beef round	Fried in open pan	15	1	40	30
2. Beef liver	Fried in open pan	10–15	1	40	35
3. Beef liver	Fried, covered pan	10	16	94	75
4. Beef heart	Steamed	30	93	190	210
5. Beef heart	Steamed	30	50	100	150
6. Pork loin	Fried in open pan	10–15	0	40	29
7. Pork loin	Fried in open pan	10–15	0	45	28
8. Pork loin	Fried in open pan	10–15	0	68	43
9. Bacon	Fried in open pan	5	0	41	17
10. Bacon	Fried in open pan	3–5	0	22	13
11. Ham	Fried in open pan	15	0	54	30
12. Ham	Fried in open pan	15	0	59	37
13. Veal chop	Fried in open pan	10–15	0	42	30
14. Veal chop	Steamed	30	84	170	180
15. Lamb leg	Roasted, 300° F.	120	0	690	550
16. Mutton shoulder	Steamed	30	66	133	199
17. Chicken leg	Fried in open pan	10–15	0	41	35
18. Chicken leg	Fried in open pan	10–15	5	69	46
19. Chicken breast	Fried in open pan	10–15	0	31	29
20. Chicken breast	Fried in open pan	10–15	0	31	29
21. Salmon	Fried in open pan	20	3	111	97
22. Halibut	Fried in open pan	7–10	2	126	104
23. Halibut	Steamed	30	0	5	5
Vegetables					
24. Beets	Boiled, covered kettle	40	266	133	124
25. Cooking water					140
26. Beets	Steamed	40	190	380	570
27. Beet tops	Steamed	10	20	40	60
28. Cabbage	Steamed	30	25	100	125
29. Carrots	Steamed	30	25	50	75
30. Cauliflower	Steamed	20	120	120	240
31. Cauliflower	Steamed	30	50	100	150
32. Onions	Fried in open pan	20	29	208	53
33. Potatoes	Boiled, covered kettle	30	200	190	200
34. Cooking water					190
35. Potatoes	Boiled, covered kettle	20	360	180	210
36. Cooking water					210
37. Lima beans	Steamed	60	10	5	15
38. Okra	Steamed	20	15	30	45
39. Rice	Steamed	25	60	30	90
40. Sauerkraut	Steamed	30	0	5	5
41. Spinach	Steamed	10	50	100	150
42. Split peas	Steamed	40	100	50	150
43. Sweet potatoes	Baked	45–60	0	2200	1700
44. Turnips	Boiled, covered kettle	40	114	114	118
45. Cooking water					90
46. Turnips	Steamed	20	22	22	44
Fruit					
47. Apples	Boiled, open kettle	20–30	1300	3400	2300
Eggs and milk					
48. Eggs	Scrambled, open pan	10–15	8	150	139
49. Eggs	Scrambled, open pan	10–15	1	50	46
50. Eggs	Baked	30	0	100	100
51. Eggs	Scrambled, covered pan	5	0	100	94
52. Milk	Heated, glass dish	30	0	100	100
53. Milk	Heated, open metal pan	15	0	100	100

[1] The numbers in this column correspond to those in the first column of table 2.

TABLE 2 *Losses of certain B vitamins during cooking*[1]

FOODS	RIBOFLAVIN			NICOTINIC ACID			PANTOTHENIC ACID		
	Total content		Loss	Total content		Loss	Total content		Loss
	Before	After		Before	After		Before	After	
Meats	µg.	µg.	%	µg.	µg.	%	µg.	µg.	%
1.[2] Beef round	88	69	22	1800	1700	5.5	190	130	32
2. Beef liver	1100	1300	0	4700	5200	0	2200	1900	14
3. Beef liver	2400	2300	4.2	14000	14000	0	8600	7400	14
4. Beef heart	1700	1700	0	15000	13000	13	3400	1900	44
5. Beef heart	890	950	0	8700	7400	15	2000	1400	30
6. Pork loin	130	87	33	1500	410	72	280	200	29
7. Pork loin	90	75	17	2300	1500	35	260	190	27
8. Pork loin	100	72	28	6200	4200	32	320	200	37
9. Bacon	43	51	0				110	110	0
10. Bacon	69	63	8.7	920	940	0	220	170	23
11. Ham	140	110	21	1800	1700	5.5	180	220	0
12. Ham	150	125	17	1900	2200	0	390	390	0
13. Veal chop	59	59	0	3000	2600	13	110	94	14
14. Veal chop	380	400	0	1200	1100	8.5			
15. Lamb leg	1700	1700	0	52000	42000	19	4100	3600	12
16. Mutton shoulder	330	330	0	5300	5300	0	570	570	0
17. Chicken leg	110	110	0	1800	1600	11	320	250	22
18. Chicken leg	170	160	5.9	2200	1800	18	360	240	33
19. Chicken breast	40	40	0	2900	2800	3.4	180	140	22
20. Chicken breast				3300	3400		170	160	5
21. Salmon	160	160	0	7100	6600	7.0	730	740	0
22. Halibut	110	86	22	9400	9200	2.1	140	140	0
23. Halibut	2.2	2.2	0	710	670	5.6	9.5	9.5	0
Vegetables									
24. Beets	51	42	18	740	340	54	170	130	24
25. Cooking water		10	0[3]		270	18[3]		9	18
26. Beets	230	230	0	2300	890	61	420	260	38
27. Beet tops	86	74	14	240	230	4.2	56	36	36
28. Cabbage	57	48	16	210	170	19	180	130	28
29. Carrots									
30. Cauliflower	150	130	13	780	610	22	970	970	0
31. Cauliflower	120	100	17	530	550	0	1000	940	6
32. Onions	50	37	26						
33. Potatoes	95	86	9.4	1600	1100	31			
34. Cooking water		14	0[3]		490	0.6[3]			
35. Potatoes	92	52	43	2600	1900	27			
36. Cooking water		40	0[3]		700	0[3]			
37. Lima beans	6.6	6.8	0	49	57	0	42	31	26
38. Okra	32	39	0	210	210	0	69	81	0
39. Rice	16	18	0	170	220	0	110	110	0
40. Sauerkraut	2.0	1.9	5.0	8.5	8.0	5.9	2.7	2.7	0
41. Spinach	220	160	27	510	430	16	160	150	6
42. Split peas	88	93	0						
43. Sweet potatoes	910	790	13	9980	9500	4.8			
44. Turnips	48	43	10	1100	660	40	160	94	41
45. Cooking water		8	0[3]		440	0[3]		54	7
46. Turnips				15	16	0			
Fruit									
47. Apples	580	430	25						
Eggs and milk									
48. Eggs	655	341	48						
49. Eggs	205	160	22						
50. Eggs	360	380	0	67	50	25	1400	1500	0
51. Eggs	260	380	0						
52. Milk	166	87	48						
53. Milk	166	130	22						

[1] The amounts of respective vitamins given in this table are those found in the number of gram food analysed shown in the last two columns of table 1.

Losses of certain B vitamins during cooking [1] TABLE 2

BIOTIN			INOSITOL			FOLIC ACID		
Total content		Loss	Total content		Loss	Total content		Loss
Before	After		Before	After		Before	After	
μg.	μg.	%	mg.	mg.	%	μg.	μg.	%
0.88	0.33	63	18	16	11	42	8.7	79
45	18	60	22	15	32	130	110	15
						540	630	0
25	11	56	420	420	0	11	3.6	67
4.9	1.5	69	290	330	0	160	12	92
2.2	1.9	14	18	13	28	26	8.0	69
1.7	0.62	64	20	13	35	61	3.2	95
1.4	0.47	66	25	20	20	57	5.4	91
3.0	2.0	33	18	7.5	58	25	1.9	92
1.7	1.4	18	14	12	14	35	2.6	91
3.2	1.8	44	17	18	0	31	3.9	87
2.4	1.8	25	34	27	21	70	4.2	94
0.59	0.30	34	15	16	0	69	29	58
3.4	2.0	41	54	32	39	160	55	66
15	15	0	400	310	24	800	100	88
3.6	2.4	33	68	77	0	100	31	69
4.6	1.3	72				61	22	64
5.7	3.7	35	26	22	15			
2.0	1.4	30	10	10	0			
1.5	0.84	43	21	14	33	63	21	67
5.9	4.5	24	19	19	0	98	37	62
12	8.4	30	24	18	25	67	36	46
0.33	0.32	3	.7	.7	0	5.0	1.3	74
						120	7	94
							5	90 [2]
1.1	1.1	0	80	43	46	170	50	71
1.1	1.0	9.1	8.3	3.9	54	84	22	74
2.4	2.8	0	95	62	35	65	5	92
			24	24	0	48	1.2	97
23	20	13	96	93	3.1	190	23	88
14	10	28	110	60	46	110	34	69
						190	14	93
							13	86 [2]
						150	6	96
							4	93 [2]
0.49	0.37	24	8.4	3.3	61	17	1.6	91
1.7	1.8	0	16	16	0	16	4.5	72
0.39	0.48	0	7.9	3.1	61	26	2.1	92
0.095	0.11	0	1.5	1.2	20	0.93	0.93	0
5.9	5.1	14	27	20	26	170	27	84
9.2	7.3	21	160	7.8	95			
						130	6	95
							8	89 [2]
0.46	0.51	0	10	11	0	11	1.5	86
			19	20	0	110	38	65
4.3	4.5	0				110	76	31

[1] The numbers in this column correspond to those in the first column of table 1.
[2] Cooking waters assayed separately and included with food samples.

Riboflavin

As has been pointed out previously (Williams and Cheldelin, '42), riboflavin losses during cooking tend to be greatest in the presence of light. Large losses, up to 48%, were incurred in the cooking of eggs, milk and pork chops.

Riboflavin losses sustained in the absence of light are, on the other hand, remarkably small. Steaming of meats or vegetables results, for the most part, in small or negligible losses of this vitamin. Similar observations have been made for meats by McIntire and coworkers ('43). The thermostability of riboflavin is indicated by the very slight destruction in baking sweet potatoes and roasting lamb. The contrast between losses in the presence and absence of light is especially marked for halibut, eggs and milk.

The opacity of many foods tends to prevent excessive destruction of riboflavin even in the light. Bacon, for example, fried for a relatively short time, retains essentially all of its riboflavin.

The apparent gains in riboflavin found with some samples are probably not significant. Substantial gains (greater than 10%) are often recorded with materials of relatively low potency where the accuracy of the assay method is poorest. In any case the assay method is probably accurate only to $\pm$ 10%. This qualification applies not only to riboflavin assays but to the others as well.

Nicotinic acid

Nicotinic acid is known to be quite stable to heat. Losses in cooking would therefore be expected to be slight. This is true for most of the samples listed in table 2, with the exception of pork chops, eggs and beets.

The small losses observed for meats are markedly lower than those reported by Waisman and Elvehjem ('41) and Dann and Handler ('42), using chemical methods of assay, although McIntire, et al. ('43) observed only slight losses in cooking of pork when microbiological assays were used. Since the results were obtained for different samples the reason for the

discrepancies is not apparent. It is possible that chemical changes in the antipellagric factor which accompany heating may not be reflected in the microbiological assay.

Pantothenic acid

Pantothenic acid losses are seen from table 2, to be moderate to slight among vegetables and eggs, as well as among such meats as bacon, ham, mutton and fish which are here subjected to relatively mild heat treatment. Beef heart and beets are exceptions. Frying of beef or pork for longer periods of time results in loss of about one third of the vitamin present. The results with meats are in general agreement with the data of Waisman and Elvehjem ('41), although the present losses are somewhat smaller for pork.

Biotin

Biotin losses among vegetables are moderate, ranging from 28% in steamed cauliflower to no loss in beets, cabbage, okra, rice, sauerkraut, and turnips. There is likewise no loss of biotin in frying eggs.

Losses in biotin among meats are generally higher, reaching 72% for fried chicken leg. Severity of heat treatment does not seem to be directly responsible for increased losses, since beef heart loses about 60% by steaming 30 minutes, whereas roasting of lamb at 150° C. for 2 hours results in practically no loss of biotin.

Inositol

Inositol losses among meats are quite variable, but are moderate in most cases. In general smaller losses are observed among samples which have been subjected to relatively mild treatment such as steaming (mutton, heart, and halibut). The greatest loss (58%) is found for one sample of fried bacon.

In contrast to most of the other B vitamins, inositol losses in cooking of vegetables are often much greater than those encountered for meats. Losses are especially high among the

legumes, with split peas losing 95% of their inositol content. In view of the stability of inositol toward heat, this is surprising especially since the severity of heat treatment of the vegetables tested was much less than that for most of the meats. It seems likely, therefore, that the losses observed may be due to "binding" of the vitamins in various tissue combinations rather than to thermal destruction.

Folic acid

Of all the vitamins studied, folic acid showed the greatest loss due to cooking. With the exception of liver and sauerkraut, which retain the bulk of their folic acid content, losses among meats range from 46% in halibut to 95% in pork chops; losses in vegetables range from 69% in cauliflower to 97% in carrots.

The reasons for such large losses are not known. Although the vitamin is unstable to light, it is not readily destroyed by autoclaving in the dark in the pH range common to most foods. It seems likely that folic acid may become "bound" in tissues during cooking. This may be investigated further at a later time.

SUMMARY

Cooking losses as determined with microorganisms, have been determined in thirty foods for riboflavin, nicotinic acid, pantothenic acid, biotin, inositol and folic acid. These are summarized below.

VITAMIN	DEGREE OF LOSS DURING COOKING
Riboflavin —	Destroyed in variable amounts in presence of light; negligible losses in the dark.
Nicotinic acid —	Generally slight.
Pantothenic acid —	Moderate to slight in vegetables; somewhat larger (up to one third) in meats.
Biotin —	Moderate to negligible in vegetables; quite high in meats (up to 72%).
Inositol —	Generally moderate among meats, with steamed samples only slight; often very great (up to 95%) in vegetables, especially in legumes.
Folic acid —	Very great for most foods.

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM IN THE CHICK

II. RELATIVE ANTIRACHITIC EFFECTIVENESS OF VITAMINS D_2 AND D_3 AND DIHYDROTACHYSTEROL ADMINISTERED PARENTERALLY [1]

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In a previous publication McChesney ('43b) reported a detailed study of the effects of vitamins D_2 and D_3 and dihydrotachysterol on the calcium and phosphorus metabolism of the chick. The various preparations were administered orally in corn oil, or intramuscularly in either propylene glycol or corn oil. Since the number of chicks used in the study was limited, and the vitamin dosages were either of the same unit value (as determined in the albino rat), or were intended to be antirachitically equivalent for the chick, too few points on the curve relating dose and response were established to permit anything better than a rough estimate of the relative effectiveness of the different preparations by the various routes of administration. The data obtained from these experiments were of sufficient interest, however, to warrant further studies of the three preparations administered by several parenteral routes. Under such conditions, differences in response due to failure of absorption from, or destruction in, the digestive tract may be entirely eliminated from consideration.

The objective of the study as a whole has been to secure information which would serve to explain the phenomenon

[1] Presented before the Division of Biological Chemistry of the American Chemical Society at Detroit, April 13, 1943.

first reported by Hess and Supplee ('30); namely, that vitamin D_2 is considerably less effective on a unit basis in the chick than natural vitamin D. In this paper an attempt has been made to establish so far as possible definite ratios of antirachitic effectiveness for the three preparations, administered either in propylene glycol or in oil, and by several parenteral routes. The ratios obtained from administration of the vitamin supplements in propylene glycol should, a priori, be of particular interest since this vehicle is readily absorbed in the tissues and does not remain in situ for long periods of time as does oil. In addition, propylene glycol may be injected intravenously. This affords an excellent means of transporting the vitamin to its site of utilization without passing it through the digestive tract or other tissues. Utilization should be as complete by this route as can be accomplished by any route of administration.

EXPERIMENTAL

Male White Leghorn chicks were received in the laboratory on the second day of life. They were immediately offered water and the vitamin D-free diet previously described (Remp and Marshall, '38). From this point forward, with the exception of the vitamin supplementation, they were managed as if a vitamin D assay were being conducted according to the procedure outlined by Massengale and Bills ('36). The birds were divided into groups of 10 to 12 on the fourteenth day of life, the groups having equal average weights. The vitamin supplements were administered on the seventeenth, twenty-fourth, thirty-first, and thirty-eighth days of life; each chick received on these days an injection of 0.1 ml. into each breast muscle (total of 0.2 ml. per week) except that the chicks which received the intravenous or intraperitoneal injections received only 0.1 ml. The intravenous injections were made into the wing or leg vein.[2] On the forty-fifth day of life all the surviving chickens were sacrificed and both tibiae were removed.

[2] The author is indebted to Mr. Kimball D. Sprague for making these injections.

The bones were prepared for ashing in the manner recommended by Massengale and Bills. The data to be reported comprise three separate experiments, all conducted according to this general plan.

Experiment 1

This experiment was designed to compare the antirachitic effectiveness of the three preparations by the intramuscular route in propylene glycol and in oil. The relative effectiveness of propylene glycol solutions of vitamin D_3, administered intramuscularly, intravenously, and intraperitoneally was also studied. The pooled left tibiae obtained from each group at the end of the experiment were ashed. In certain of the more critical groups the right tibiae were also ashed individually in order that the probable error of the mean could be calculated. The data are given in table 1.

Experiment 2

The results of experiment 1 indicated that vitamin D_3 in propylene glycol is utilized to the same extent whether administered by the intramuscular or intravenous routes. These routes may therefore be considered, for practical purposes, to permit complete utilization of the vitamin, since the intramuscular injections give results which are superior to either the oral (McChesney, '43b) or the intraperitoneal routes (vide infra). These considerations made it of interest to investigate also the relative utilization of vitamin D_2 and dihydrotachysterol when administered by the intramuscular and intravenous routes in propylene glycol. Furthermore, additional data on the antirachitic effectiveness of these two preparations when given by the intramuscular route was desirable since the doses given in experiment 1 did not cover a sufficiently wide range.

This experiment was conducted in exactly the same manner as the preceding one except that the surviving chicks were

TABLE 1

Relative effectiveness of vitamins D_2, D_3 and dihydrotachysterol administered parenterally.

GROUP NO.	VEHICLE	TYPE OF INJECTION	PREPARATION	U.S.P. UNITS PER WEEK	PERCENT WEIGHT GAIN [2]	PERCENT MORTALITY	AVERAGE PERCENT BONE ASH		EFFECTIVE UNITS PER WEEK [6]	
							Left tibiae [3]	Right tibiae [4]		
1	Propylene glycol	Intramuscular	Vitamin D_2	25	99	33.3	30.39	30.22 ± 0.45 [5]	..	
2	Propylene glycol	Intramuscular	Vitamin D_2	50	127	8.3	33.20	32.84 ± 0.30	1.6	
3	Propylene glycol	Intramuscular	Vitamin D_2	100	189	8.3	39.10	38.20 ± 0.61	7.2	
4	Propylene glycol	Intramuscular	Vitamin D_3	5	168	0	36.21	37.14 ± 0.32	5	Reference groups
5	Propylene glycol	Intramuscular	Vitamin D_3	10	185	0	42.75	42.03 ± 0.61	10	Reference groups
6	Propylene glycol	Intramuscular	Vitamin D_3	20	200	0	45.20	45.38 ± 0.26	20	Reference groups
7	Propylene glycol	Intramuscular	Dihydrotachysterol	1	100	0	33.33	33.94 ± 0.60	1.7	
8	Propylene glycol	Intramuscular	Dihydrotachysterol	2	110	0	35.00	34.13 ± 0.31	3.6	
9	Propylene glycol	Intramuscular	Dihydrotachysterol	4	152	0	35.32	35.45 ± 0.47	4.0	
10	Propylene glycol	Intraperitoneal	Vitamin D_3	10	212	0	38.83		7.0	
11	Propylene glycol	Intravenous	Vitamin D_3	10	217	8.3	42.20		9.6	
12	Corn oil	Intramuscular	Vitamin D_2	500	127	25.	32.09	32.90 ± 0.55	0.2	
13	Corn oil	Intramuscular	Vitamin D_2	1000	134	16.6	34.37	35.12 ± 0.48	2.9	
14	Corn oil	Intramuscular	Vitamin D_3	100	212	0	40.27		8.1	
15	Corn oil	Intramuscular	Vitamin D_3	200	195	0	46.19		23 (Approx.)	
16	Corn oil	Intramuscular	Dihydrotachysterol	20 [1]	151	8.3	33.82		2.2	
17	Corn oil	Intramuscular	Dihydrotachysterol	40 [1]	142	0	36.74		5.4	
18	None	.....	None	0	127	8.3	31.91	32.20 ± 0.27	0	

[1] One ml. of the commercial 0.5% solution assays 110 to 120 U.S.P. units in the albino rat, and contains about 1.25 mg. of the active principle.

[2] Based on the weights of the 45th day of life as compared to those of the 17th day of life.

[3] Ashed in groups.

[4] Ashed individually.

[5] Probable error of the mean.

[6] Calculated as vitamin D_3; based on the composite group ash values obtained from intramuscular administration in propylene glycol. (Groups 4, 5, 6, and 18).

sacrificed on the forty-third day of life instead of the forty-fifth. This change was considered advisable because in certain groups the birds were dying rather rapidly, and in others marked leg weakness indicated a severe grade of rickets, suggesting that the degree of depletion was greater than in the other experiments.

Individual bone ash determinations were made on the left tibiae, but the results in terms of vitamin D potencies are based on the composite of the groups (total weight of bone ash for the group divided by total weight of moisture and fat-free bones times 100), since this procedure was used in evaluating the results of experiment 1. This has the effect of placing a greater weight on the ash values of the larger birds. The results are given in table 2.

Experiment 3

The amounts of vitamin D_2 administered in oil in experiment 1 were insufficient to permit more than a slight degree of calcification. Therefore the effects of vitamin D_2 were investigated over a much wider dosage range in order to establish a more definite ratio between the requirements for vitamin D_2 in oil when given by the intramuscular and oral routes. Doses ranging from 500 to 16,000 units of D_2 per week were given intramuscularly in 0.2 ml. of corn oil, and their effects were compared with that of 1000 units per week orally (as a single dose) in 0.2 ml. of the same vehicle. The experimental procedures were the same as in experiments 1 and 2. Careful mortality records were kept, since the data reported in the earlier publication indicated greater mortality among chicks receiving 96 units per week of vitamin D_2 intramuscularly in oil than among negative control chicks injected with corn oil only. The symptoms observed suggested that this vitamin supplement aggravated the rickets rather than alleviated it. The results of this experiment are given in table 3.

TABLE 2

Utilization of vitamin D_3 and dihydrotachysterol when administered parenterally in propylene glycol.

GROUP	PREPARATION	ROUTE OF ADMINISTRATION	U.S.P. UNITS PER WEEK	AVERAGE PERCENT WEIGHT GAIN [1]	PERCENT MORTALITY	PERCENT BONE ASH		UNITS OF VITAMIN D_3 (CALCULATED FROM COMPOSITE BONE ASH)	
						Average	Composite		
1	Vitamin D_3	Intramuscular	60	109	55	31.43 ± 0.91 [3]	31.64	60	Reference groups
2	Vitamin D_3	Intramuscular	125	126	0	35.11 ± 0.51	35.50	125	Reference groups
3	Vitamin D_3	Intramuscular	250	195	0	37.26 ± 0.80	37.74	250	Reference groups
4	Vitamin D_3	Intravenous	125	124	36 [2]	36.23 ± 0.85	36.40	175	
5	Dihydrotachysterol	Intramuscular	4	116	18	32.49 ± 0.48	32.70	80	
6	Dihydrotachysterol	Intramuscular	7	134	0	36.12 ± 0.49	36.98	215	
7	Dihydrotachysterol	Intramuscular	10	176	9	38.54 ± 0.92	39.24	300 (Approx.)	
8	Dihydrotachysterol	Intravenous	7	104	27 [2]	34.76 ± 0.45	34.90	130	
9	Vitamin D_3	Intramuscular	10	189	0	36.98 ± 0.34	36.65	190	
10	Propylene gycol	Intramuscular	0	44	27	29.10 ± 0.53	28.62	0	

[1] All chicks were sacrificed on the 43rd day of life. Percent gain refers to the average gain of the group between the 17th and 43rd days of life.

[2] Several chicks in these groups were killed accidentally while the injections were being made; they did not die as a result of vitamin deficiency.

[3] Probable error of the mean.

TABLE 3

Relative antirachitic effectiveness of vitamin D_3 given to chicks intramuscularly and orally in corn oil.

GROUP	ROUTE OF ADMINISTRATION	U.S.P. UNITS PER WEEK	AVERAGE PERCENT WEIGHT GAIN [2]	PERCENT MORTALITY	AVERAGE LENGTH OF LIFE, DAYS [1]	PERCENT BONE ASH	
						Extreme range	Average [3]
1	Intramuscular	0	63	40	43.0	28.1–36.9	32.61 ± 0.92 [3]
2	Intramuscular	500	46	50	40.8	27.9–33.9	30.76 ± 0.94
3	Intramuscular	1000	115	30	42.1	32.8–40.8	35.30 ± 0.80
4	Intramuscular	2000	140	20	41.2	29.3–46.5	36.70 ± 1.39
5	Intramuscular	4000	108	20	41.1	30.2–45.7	38.04 ± 1.56
6	Intramuscular	8000	163	0	45.0	31.6–46.4	38.28 ± 1.26
7	Intramuscular	16000	235	10	43.5	34.9–47.1	43.07 ± 0.92
8	Oral	1000	169	0	45.0	35.7–42.1	38.56 ± 0.53

[1] All chicks were sacrificed on the 45th day of life.
[2] Ashed individually.
[3] Probable error of the mean.

DISCUSSION

While intramuscular and intravenous injections of vitamin D_3 in propylene glycol are equally effective, this may not be true of vitamin D_2 or of dihydrotachysterol. The data indicate that utilization of vitamin D_2 following intravenous injection was apparently 40% superior to that noted following intramuscular injections of the same supplement. However, when the probable errors of the means are taken into account, the difference between these groups (table 2, groups 2 and 4) is not statistically significant. Dihydrotachysterol appears to be about 40% less effective by the intravenous route than by the intramuscular route and in this case the differences noted are statistically significant, (table 2, groups 6 and 8). In both cases, however, the differences do not sufficiently exceed the experimental error to justify extensive speculation as to their cause unless they can be confirmed. In the one case in which intraperitoneal injections were given (table 1, group 10) the results show that there is probably some inactivation before the material reaches the blood stream, since the calcification was considerably inferior to that given by the same unit dose administered intramuscularly.

Utilization following intramuscular injections of vitamins D_2 and D_3 in propylene glycol is considerably superior to that noted after oral administration in oil. The first paper of this series showed that under the test conditions used the oral administration of 21 units of vitamin D_3 per week in oil gave a bone ash value of 39.6%, while the intramuscular injection of 20 units per week in propylene glycol gave a bone ash value of 45.6%. This difference clearly indicates that the utilization of the vitamin after intramuscular injection is two or two and one-half times superior to that obtained by the oral route (see data of Massengale and Bills, op. cit., p. 432).[3]

[3] These authors found that it required 6 units of natural vitamin D per 100 gm. of diet to produce a bone ash value of 39.6%, and 15.5 units per 100 gm. to produce a bone ash value of 45.6%. This indicates that the intramuscular dosage is about 1.5 times more effective than the oral. In this connection, our unpublished laboratory data on the albino rat show that vitamin D_3 in propylene glycol is about 50% more effective when injected intramuscularly than when given orally.

Although dihydrotachysterol is about 300% more effective per U.S.P. unit than vitamin D_3 when they are given orally to the chick, it is at the most 60% more effective (probably less) when they are given intramuscularly in either propylene glycol or oil. The most logical interpretation of this phenomenon is that when administered by way of the digestive tract the dihydrotachysterol is converted to a substance of considerably greater antirachitic value.

Vitamin D_3, administered intramuscularly in oil, is about one-fourth as effective in promoting calcification as when given orally, and about one-tenth as effective as when given intramuscularly in propylene glycol. Of vitamin D_2 and dihydrotachysterol, injected intramuscularly in oil, about one-ninth and one-twelfth, respectively, appear to become effective within the experimental period as compared to oral dosages. In the latter case, for reasons pointed out above, the fraction assigned is probably meaningless. The responses to vitamin D_2 administered intramuscularly in oil were very erratic, indicating large individual differences in absorption.

The peculiar phenomenon noted earlier, namely, that when doses of vitamin D_2 (equal in magnitude to about 10% of the curative dose) are given intramuscularly, they appear to exert an anticalcifying effect, has again been observed (table 1, group 1; table 3, group 2). Further study will be required to determine the significance of this observation.

In order to summarize in a convenient form the data obtained in these experiments, a table of the relative effectiveness of the different preparations administered by the several routes has been drawn up. It must be kept in mind that the preparation of such a table involves the carrying of results of one experiment over into another; this can be done exactly only if the negative controls give comparable results and certain positive responses are established as reference points in each experiment. Furthermore, quantitative comparisons which apply at the bone ash level selected would not necessarily apply at higher or lower levels. Because of the nature of the data available, all quantities have been evaluated in terms of those dosages required to produce a final bone

TABLE 4

Estimated requirements of the chick for three activated sterols in different vehicles and by several routes of administration.

PREPARATION	VEHICLE	ROUTE OF ADMINISTRATION	U.S.P. UNITS REQUIRED PER WEEK [1]
Vitamin D_3	Propylene glycol	Intramuscular	9 [2]
	Propylene glycol	Intravenous	9 [3]
	Propylene glycol	Intraperitoneal	13 [4]
	Corn oil	Intramuscular	90 [5]
	Corn oil	Oral	24 [6]
Vitamin D_2	Corn oil	Oral	850 [6]
	Corn oil	Intramuscular	8,000 [7]
	Propylene glycol	Intramuscular	150 [8]
	Propylene glycol	Intravenous	110 [9]
Dihydrotachysterol	Propylene glycol	Intramuscular	6 [10]
	Propylene glycol	Intravenous	10 [11]
	Corn oil	Intramuscular	75 [12]
	Corn oil	Oral	6 [6]

[1] To produce a standard response of 41–42% bone ash (average) under the experimental conditions, the negative controls at the same time having a bone ash of 31–32%.

[2] The table was compiled in the following manner. The values of the reference groups in table 1 (groups 4, 5, 6, and 18) were plotted on graph paper. From the graph it was determined that 9 units of vitamin D_3 administered intramuscularly in propylene glycol would have given the "standard response" (in this case, 41.9% bone ash since the negative controls gave 31.9% ash.) The remainder of the table is based on this one figure. Since all of the responses in table 1 were evaluated in terms of "effective units," using the response to vitamin D_3 administered intramuscularly in propylene glycol as a basis, a table of equivalences is readily computed as given in detail below. The nearest whole or round number is used for obvious reasons.

[3] From table 1, groups 5 and 11; i.e., $9 \times \frac{10}{9.6} = 9$.

[4] From table 1, groups 5 and 10; i.e., $9 \times \frac{10}{7} = 13$.

[5] From table 1, group 5 and the average of groups 14 and 15 i.e., $9 \times \frac{100}{10} = 90$.

[6] From data presented in the first paper of this series.

[7] From table 3, groups 6, 7 and 8; i.e., the intramuscular dose must be 9–10 times the oral dose in order to have an equivalent antirachitic effect; $9 \times 850 = 8000$ approximately.

[8] From table 1, group 3 and table 2, group 9, which place the required dose of vitamin D_3 at from 14 to 19 times the dose of D_2.

[9] From table 2, groups 2 and 4, assuming the value obtained in footnote 8 to be correct; i.e., $150 \times \frac{125}{175} = 110$.

[10] From table 2, groups 7 and 9; i.e., (the reference value of) $9 \times \frac{190}{300} = 6$ approx.

[11] From table 2, groups 6 and 8; i.e., (from footnote 10) $6 \times \frac{215}{130} = 10$.

[12] From table 1, groups 16 and 17; i.e., (the reference value of) $9 \times \frac{20}{2.2} = 82$ or $9 \times \frac{40}{5.4} = 67$.

ash value (when the bones are ashed in groups) of 41–42%. The table has been based on Experiment 1, with the application of ratios of effectiveness derived from the other experiments where necessary to complete the table. In view of these considerations no claim is made as to the reproducibility of the numerical values given, but it is expected that the ratios of effectiveness derived from them would generally be reproducible within $\pm$ 25%. The data are given in table 4.

SUMMARY

The relative effectiveness of vitamin D_2 and D_3 and dihydrotachysterol when administered parenterally to the chick in propylene glycol and corn oil has been studied. Rather definite requirements have been established for the different preparations by the several routes of administration and are presented in the form of a table. Although the weekly oral requirements (as defined herein) of the three preparations administered in oil are: vitamin D_3, 24 units; vitamin D_2, 850 units; and dihydrotachysterol, 6 units (i.e., in the proportions of 1:35:0.25), the intramuscular requirements in propylene glycol (in the same order) are 9,150, and 6 units (i.e., in the proportions of 1:16:0.7) and the intravenous requirements (in the same order) are 9,110, and 10 units (i.e., in the proportions of 1:12:1.1). Thus although dihydrotachysterol is 300% more effective than vitamin D_3 by the oral route, it averages only about 20% more effective by the parenteral routes. The data on relative oral and parenteral utilization of vitamins D_2 and D_3 indicate that the latter is somewhat better absorbed from the digestive tract.

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THE EFFECT OF THIAMINE, RIBOFLAVIN OR PYRIDOXINE DEFICIENCY ON THE INTESTINAL ABSORPTION OF GALACTOSE IN THE RAT[1]

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Previous reports from this laboratory (Free and Leonards, '42; Leonards and Free, '43) have indicated that the rate of intestinal absorption of galactose of pair-fed litter-mate control rats was 35% more than that of animals deficient in the vitamin B complex and 15% more than that of animals deficient in pantothenic acid. The present report describes studies of the effect of deficiency of other members of the vitamin B complex on intestinal absorption.

METHODS

Three groups of young albino and piebald rats of both sexes were placed on a diet consisting of casein, sugar, starch, cottonseed oil, cod liver oil and choline chloride identical with that previously used in this type of study (Leonards and Free, '43). The daily vitamin B supplements per rat were thiamine 40 μg.; riboflavin 25 μg.; pyridoxine 20 μg.; and calcium pantothenate 100 μg.[2] By the omission of the proper

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[2] We are indebted to Merck and Company, Rahway, N. J., for generous supplies of the vitamins employed in this study.

supplement one group of rats received a diet deficient in thiamine, another group in riboflavin and the third group in pyridoxine. Each experimental animal was "paired" with a litter-mate of the same sex which served as a control. The control animals received all of the above vitamin supplements. The food intake of the control member of the pair was regulated so that its weight was kept nearly the same as that of the experimental member.

The thiamine deficient rats were kept on the diet for 38 to 45 days at which time the animals showed deficiency symptoms similar to those observed in our previous study on vitamin B complex deficiency (Free and Leonards, '42). At this time the studies of intestinal absorption were carried out. Polyneuritis was not observed in any of the rats.

The animals deficient in riboflavin grew very slowly and ceased gaining weight after about 3 weeks. At this time vascularization of the cornea was observed in six of the twenty-six experimental animals. The studies of intestinal absorption were carried out after 38 to 48 days at which time the animals had started to lose weight.

Measurements of the rate of intestinal absorption were carried out on the pyridoxine deficient rats after they had been on the diet for 40 days. The only deficiency symptom which could be observed was the failure to gain weight. The technics for the measurement of intestinal absorption and blood galactose level employed in this study were identical with those already described (Free and Leonards, '42). Exactly 1 hour after ingesting 6 gm. per kilogram of galactose the animals were sacrificed and the amounts of the sugar remaining in the stomach and intestines were determined separately. The galactose absorbed is the difference between the amount of sugar ingested and that found in the gastrointestinal tract. The coefficient of absorption is a value expressing the milligrams of galactose absorbed per 100 gm. of body weight per hour. The level of galactose in the blood was also determined at the time the animals were sacrificed and is used as an indirect measure of the rate of intestinal absorption of the sugar.

RESULTS AND DISCUSSION

Thiamine deficiency

The results of the absorption measurements on fourteen pairs of rats in which the experimental member of each pair was deprived of thiamine are shown in table 1. In all of the tables albino rats are designated by W and piebald animals by P. The coefficient of absorption is a value expressing the milligrams of galactose absorbed per 100 gm. of body weight per hour. The average coefficient of the thiamine deficient animals was 218 while the controls averaged 361 mg. per 100 gm. per hour. Although there is considerable variation in absorption coefficients among the individual animals, it is significant that in all but one of the fourteen pairs of rats that were studied the coefficient of the control member was greater than that of the thiamine deficient rat. It is also significant that the impairment of intestinal absorption in thiamine deficiency is even greater than the impairment which we have previously observed (Free and Leonards, '42) when rats were deficient in all the members of the vitamin B complex. In both the deficient and control animals a considerable percentage of the ingested galactose remained in the stomach, amounting to 41% in the deficient animals and 27% in the controls. However, the intestines of the deficient rats contained more galactose than the controls; and since this is the sugar available for absorption, gastric evacuation probably was not an important factor in causing the thiamine deficient animals to exhibit a decreased rate of intestinal absorption. The results of the present study do not indicate the mechanism by which the thiamine deficiency exerts its effect on intestinal absorption.

The blood galactose levels of the deficient rats 1 hour after the ingestion of the sugar averaged 427 mg. per 100 ml. while the controls averaged 566 mg. per 100 ml. In each of the thirteen pairs in which the absorption coefficient of the deficient member was less than that of the control, the same result prevailed for the blood galactose level. Thus it is again evident that the blood galactose level is an indirect measure of the

TABLE 1

The effect of thiamine deficiency on absorp ion coefficients and blood galactose levels.

Rat			Deficient animals						Control animals						Difference (control minus deficient)	
			Body weight		Galactose				Body weight		Galactose					
Pair	Strain	Sex	Initial	Final	In stomach [1]	In intestines [1]	Absorption coefficient	In blood	Initial	Final	In stomach [1]	In intestines [1]	Absorption coefficient	In blood	Absorption coefficient	Blood galactose
			gm.	gm.	%	%	mg./100 gm./hr.	ml.	gm.	gm.	%	%	mg./100 gm./hr.	ml.		
1	P	M	33	35	53	19	168	447	33	31	24	16	360	500	+ 192	+ 53
2	P	M	32	31	62	34	24	187	33	35	17	11	432	680	+ 408	+ 493
3	P	M	51	44	37	17	276	..	51	60	36	8	336	..	+ 60	..
4	P	M	51	49	44	20	216	..	58	54	44	14	252	..	+ 36	..
5	P	M	28	26	40	15	270	557	40	41	24	12	384	620	+ 114	+ 63
6	P	M	38	33	32	31	222	474	41	43	26	14	360	648	+ 138	+ 174
7	P	M	40	33	28	18	324	630	34	40	42	12	276	465	− 48	− 165
8	P	M	37	29	28	27	270	594	36	44	25	14	366	660	+ 96	+ 66
9	P	F	33	30	45	24	186	201	30	37	1	10	534	575	+ 348	+ 374
10	P	F	38	37	38	25	222	460	36	50	24	18	348	562	+ 126	+ 102
11	P	F	36	32	50	29	126	451	39	46	38	14	288	540	+ 162	+ 89
12	P	F	31	46	70	16	84	290	28	38	46	13	246	379	+ 162	+ 89
13	W	F	38	37	29	12	354	268	43	49	13	11	456	589	+ 102	+ 321
14	W	M	40	40	28	20	312	558	35	42	21	9	420	577	+ 108	+ 19
Average			37	36	41	22	218	427	38	44	27	13	361	566	+ 143	+ 140

[1] Fraction of ingested sugar found in the organ.

rate of absorption of the sugar. However, the blood galactose levels of the deficient rats are somewhat higher than would be predicted from the absorption coefficients, a fact indicating that the rate of removal of absorbed galactose from the blood is somewhat slower than normal. This may be due to an impairment in either the metabolism or excretion of the sugar. These findings are similar to those which we have observed in pantothenic acid deficiency (Leonards and Free, '43) and are also in accord with the work of Williams and co-workers ('40), who found a decrease in the glucose tolerance during experimental thiamine deficiency in human subjects. Elsom and co-workers ('40) also noted a decrease in glucose tolerance in a human subject rendered deficient in the vitamin B complex. Lepkovsky, Wood and Evans ('30) found an impairment in glucose tolerance of thiamine deficient rats and Pachman ('31) showed that such rats exhibit a decreased glucose tolerance when the sugar is injected intravenously.

Riboflavin deficiency

The average absorption coefficient of the riboflavin deficient animals was 352 mg. per 100 gm. per hour whereas that of their paired controls averaged 367 mg. per 100 gm. per hour. This difference is well within the experimental error. The individual results are given in table 2. There were the expected variations in absorption coefficients among the individual animals. In fifteen of the twenty-six pairs the absorption coefficient of the control member exceeded that of the deficient companion while the reverse was the case in the other eleven pairs. There is little doubt that under the conditions of these experiments a deficiency of riboflavin was without effect on the rate of absorption of galactose. These observations do not support the hypothesis that riboflavin is intimately connected with intestinal absorptive function (Sciclounoff, F. and Alphonse, P., '41; Antognini, R., '41). Beams, Free and Glenn, '41, noted that two of four cases of rosacea keratitis which responded to riboflavin therapy gave indication of decreased absorption of galactose which improved after therapy when the signs of rosacea keratitis disappeared.

TABLE 2

The effect of riboflavin deficiency on absorption coefficients and blood galactose levels.

Rat			Deficient animals						Control animals						Difference (control minus deficient)	
			Body weight		Galactose				Body weight		Galactose					
Pair	Sex	Strain	Initial	Final	In stomach[1]	In intestines[1]	Absorption coefficient	In blood	Initial	Final	In stomach[1]	In intestines[1]	Absorption coefficient	In blood	Absorption coefficient	Blood galactose
			gm.	gm.	%	%	mg./100 gm./hr.	ml.	gm.	gm.	%	%	mg./100 gm./hr.	ml.		
1	F	P	36	39	29	18	318	330	38	37	16	12	432	..	+ 114	..
2	F	P	29	25	20	21	354	598	26	28	42	17	246	460	− 108	− 138
3	F	P	35	32	6	16	468	760	36	40	18	13	414	601	− 54	− 159
4	F	P	29	32	22	16	372	506	40	35	51	11	228	423	− 144	− 83
5	M	P	37	43	14	13	438	642	37	33	30	14	336	507	− 102	− 135
6	M	P	25	27	32	6	372	598	22	29	15	8	462	657	+ 90	+ 59
7	M	P	30	33	24	22	324	492	30	35	17	54	174	425	− 150	− 67
8	M	P	27	33	46	28	156	331	30	39	34	25	246	465	+ 90	+ 134
9	M	P	25	29	54	18	168	401	30	42	25	13	372	635	+ 204	+ 234
10	M	P	42	45	27	20	318	564	30	43	28	12	360	600	+ 42	+ 36
11	F	W	29	45	20	14	396	491	27	45	23	10	402	521	+ 6	+ 30
12	F	W	32	34	26	13	366	561	26	30	5	14	486	697	+ 120	+ 136
13	F	W	37	42	15	31	324	392	34	46	9	34	342	452	+ 18	+ 60
14	F	W	32	40	12	22	396	502	32	43	40	7	318	465	− 78	− 37
15	F	W	89	77	24	15	366	631	87	77	24	12	384	735	+ 18	+ 104
16	F	W	95	90	37	10	318	640	75	88	32	9	354	618	+ 36	− 22
17	F	W	26	30	2	15	498	611	30	36	11	10	474	548	− 24	− 63
18	F	W	30	32	1	40	354	331	28	35	19	11	420	600	+ 66	+ 269
19	F	W	69	64	38	13	294	448	28	45	27	10	378	604	+ 84	+ 156
20	F	W	26	35	20	12	408	688	28	39	26	13	366	657	− 42	− 31
21	M	W	40	40	27	18	330	572	42	34	15	2	498	657	+ 168	+ 85
22	M	W	32	41	15	14	426	600	44	44	23	13	384	577	− 42	− 23
23	M	W	31	46	11	13	456	732	30	40	8	9	498	593	+ 42	− 139
24	M	W	42	50	14	13	438	577	40	47	23	12	390	550	− 48	− 27
25	M	W	23	29	16	23	366	607	27	36	34	18	288	532	− 78	− 75
26	M	W	108	112	63	14	138	282	98	112	43	8	294	524	+ 156	+ 242
Average			40	44	24	18	352	543	38	44	24	14	367	564		

[1] Fraction of ingested sugar found in the organ.

Figures for blood galactose indicate an average value of 543 mg. per 100 ml. for the deficient animals and 564 mg. per 100 ml. for the controls. In about one-half of the pairs the blood galactose level of the control member was greater than that of the deficient member, the reverse being true in the other pairs. In general the blood galactose levels corresponded to the rate of intestinal absorption as measured directly by the absorption coefficients. In only two of the twenty-six pairs (nos. 16 and 23 in table 2) did the comparison of the blood galactose levels of deficient and control members not correspond with their absorption coefficients.

Pyridoxine deficiency

The individual results of the absorption measurements of thirteen pairs of rats in which the experimental member was deprived of pyridoxine for 40 days are given in table 3. The average absorption coefficient of the deficient rats was 304 mg. per 100 gm. per hour as compared with 340 mg. per 100 gm. per hour for the controls. The average blood galactose levels were respectively 550 mg. per 100 ml. and 597 mg. per 100 ml. for the deficient and control groups. Thus as evidenced by both direct measurement of the rate of intestinal absorption and by blood galactose levels there was a slight decrease in the rate of absorption of galactose in the deficient animals. The decrease amounted to about 10%. This may perhaps be significant since in nine of the thirteen pairs the rate of absorption was less in the deficient than in the control member of the pair. It is also interesting to note that the fraction of ingested galactose remaining in the stomachs of the deficient and control animals was identical, whereas the average amount in the intestines of the deficient rats was more than in the intestines of the control rats.

It is evident that of the members of the vitamin B complex that were studied, a deficiency in thiamine resulted in the greatest degree of impairment of intestinal absorption of galactose. This may be due in part to the fact that the thiamine deficient animals were in a more severe state of deficiency.

TABLE 3

The effect of pyridoxine deficiency on absorption coefficients and blood galactose levels.

RAT			DEFICIENT ANIMALS						CONTROL ANIMALS						DIFFERENCE (CONTROL MINUS DEFICIENT)	
			Body weight		Galactose				Body weight		Galactose					
Pair	Strain	Sex	Initial	Final	In stomach [1]	In intestines [1]	Absorption coefficient	In blood	Initial	Final	In stomach [1]	In intestines [1]	Absorption coefficient	In blood	Absorption coefficient	Blood galactose
			gm.	*gm.*	%	%	*mg./100 gm./hr.*	*mg./100 ml.*	*gm.*	*gm.*	%	%	*mg./100 gm./hr.*	*mg./100 ml.*		
1	W	M	41	59	32	10	348	474	44	65	45	7	288	487	− 60	+ 13
2	W	M	44	63	39	13	288	612	40	65	43	10	282	604	− 6	− 8
3	W	M	43	60	48	23	174	418	41	60	10	11	474	781	+ 300	+ 363
4	W	M	46	68	39	10	306	621	46	67	48	10	252	514	− 54	− 107
5	W	M	45	55	22	11	402	728	34	58	36	9	330	576	− 72	− 152
6	W	M	43	61	33	10	342	576	43	61	29	8	378	697	+ 36	+ 121
7	W	M	41	60	32	19	294	563	41	66	34	6	360	577	+ 66	+ 14
8	W	M	41	57	25	21	324	460	41	61	27	12	366	612	+ 42	+ 152
9	W	M	38	60	30	25	270	415	32	53	32	8	360	585	+ 90	+ 170
10	W	M	41	51	39	15	276	585	40	52	42	10	288	581	+ 12	− 4
11	W	F	45	57	23	17	360	617	41	57	28	8	384	635	+ 24	+ 18
12	W	F	34	49	42	15	258	599	44	74	39	10	306	545	+ 48	− 54
13	W	F	35	49	34	14	312	465	31	49	27	14	354	565	+ 42	+ 100
Average			41	58	34	16	304	550	40	60	34	10	340	597		

[1] Fraction of ingested sugar found in the organ.

Under the conditions of these experiments, a deficiency of riboflavin or pyridoxine was not followed by any evidence of gross intestinal dysfunction. However, the absorption studies in these animals were made at a time when the animals began to lose weight but before the occurrence of any crisis or severe deficiency symptoms. Thus these findings do not lend themselves to a prediction of the effect of acute stages of riboflavin or pyridoxine deficiency on the intestinal absorption.

SUMMARY

Studies of the rate of intestinal absorption of galactose were carried out in rats deficient in thiamine, rats deficient in riboflavin and rats deficient in pyridoxine. Each deficient animal was paired with a litter-mate control which received adequate supplements of the missing vitamin.

The rate of intestinal absorption of galactose of pair-fed litter-mate controls averaged 66% more than that of the thiamine deficient animals, and 12% more than that of the pyridoxine deficient rats. Riboflavin deficiency had no effect on the rate of intestinal absorption of galactose in the rat.

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A STUDY OF NORMAL HUMAN REQUIREMENTS FOR ASCORBIC ACID AND CERTAIN OF ITS METABOLIC RELATIONSHIPS [1]

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The necessity for further research to determine specific nutrient needs for individuals was emphasized in the recommendations made to the President by the National Nutrition Conference for Defense ('41). Kruse ('42) discussed our need for determining optimal intakes of ascorbic acid since it is evident that a large proportion of the population show a past dietary regimen inadequate with respect to this nutrient. As recently as September, 1942, Gordon ('42) stated "the question of the daily human requirement of ascorbic acid must be considered still unsettled". Our need for more knowledge concerning optimal intake of food nutrients was recently summarized in Nutrition Reviews ('43) in the statement that a "good" diet must furnish an optimal quantity of all essential food factors. A number of investigators (Göthlin, '33, Hawley, Stephens and Anderson, '36, Widenbauer, '37, and Abbasy, Harris, Ray and Marrack, '35) who have sought to determine human requirements for ascorbic acid have obtained figures which are now recognized as the minimal intake necessary to prevent the manifestation of a

[1] Material based on a doctoral thesis submitted by Helen J. Purinton to the Graduate School, April, 1943. Aided by a grant from the Purdue Research Foundation. Present address of H.J.P., Agricultural Experiment Station, University of New Hampshire, Durham, N. H.

deficiency of this substance. It was also noted that several investigators (Gaehtgens and Werner, '37, Baumann and Rappolt, '37, Todhunter, Robbins and McIntosh, '42 and Crandon, Lund and Dill, '40) have used the term "at least" in connection with some standard daily requirement figures for ascorbic acid. Studies attempting to develop a means for determining optimal intakes of ascorbic acid are very few. The requirement figures for ascorbic acid occurring in the literature vary from 20 to 150 mg. per day, and from 0.4 mg. to 2.0 mg. per kilogram of body weight.

The principal factor contributing to our lack of knowledge concerning an optimal intake of ascorbic acid has been the disagreement among various laboratories as to the best method for determining human requirements. The use of the saturation test dose method for determining these requirements has met with success in the laboratories of several workers (Goldsmith and Ellinger, '39, Ludden and Wright, '40, Wright, Lilienfeld and McLenathan, '37, and Glazebrook and Thomson, '42). The main reason for variations in results obtained by this method seems to be due to the manner in which the test dose is administered. Several investigators have compared the validity of results obtained with oral versus intravenous administration of the test dose. Ludden and Wright ('40), Wright, Lilienfeld and McLenathan ('37), Faulkner and Taylor ('38) and Hawley and Stephens ('36) have compared the oral and intravenous technics as means of measuring absorption and utilization of a test dose. These investigators obtained more consistent results in any one individual when the test dose was administered intravenously. Their work gave evidence that, in response to a specific dose administered orally, great variations could be expected due to physiological differences in the rate of absorption and utilization.

Ludden and Wright ('40), Harris and Ray ('35), Levcowich and Batchelder ('42) and Hawley, Stephens and Anderson ('36) have determined that long time balance studies of

ascorbic acid are unnecessary for the investigation of normal requirements.

Since our knowledge as to the manner in which ascorbic acid functions is far from complete, there are no specific means at present by which optimal as contrasted to adequate intakes of ascorbic acid can be measured. The authors of this study proposed to determine an optimal utilization figure by measuring the blood concentration and urinary elimination of a relatively large number of apparently normal individuals in response to a test dose administered intravenously.

EXPERIMENTAL PROCEDURE

College students including both men and women and a few women staff members served as subjects for this study. The men were from the Department of Biochemistry and the women represented majors and instructors in the Department of Foods and Nutrition.

The subject to be tested was given a definite diet with an ascorbic acid content of 15 mg. for the males and 14 mg. for the females. For 24 hours prior to the administration of the test dose of ascorbic acid the subject was requested to continue his normal activities. The amount of water consumed throughout the diet period was not restricted. A "fasting" 24-hour urine sample was collected from the subjects upon this diet. A "fasting" sample of blood was taken before breakfast on the day of administration of the test dose. This sample was taken from the finger and was obtained in order to determine whether or not the subject being studied was to be classed in the normal or subnormal group as regards the fasting content of ascorbic acid in the body. In these studies, using the evidence of Wright, Lilienfeld and McLenathan ('37), Ludden and Wright ('40), and Thysell ('39), all subjects whose ascorbic acid content of the "fasting" plasma was 0.8 mg. per 100 ml. or above were classed as normal and those whose "fasting" plasma values were below this figure were classed as subnormal. (In the evaluation of the results obtained, these two classifications will be con-

sidered separately as well as combined, to determine the percentage of an apparently normal group of individuals who actually showed past nutritional inadequacy with respect to ascorbic acid, and to determine the requirements of individuals who gave proof of an adequate past dietary regimen.)

Although the original plans for this study called for the administration of the test dose intravenously, due to circumstances beyond our control, it was necessary in some cases to give the test dose by mouth. After the "fasting" blood sample was taken, the 500 mg. test dose of ascorbic acid was given, either orally or intravenously (500 mg. in 5 ml. physiological saline [2]). The diet for the 24 hours following administration of the test dose was exactly the same as that of the previous day. The response of the subject to the test dose was followed in the blood stream by measuring the ascorbic acid of the plasma at regular intervals until the absorption of the dose (either from the intestine or from the blood into the tissues) was indicated by a return of the plasma value to the fasting level. The response to the test dose was followed simultaneously in the urinary excretion values. The urine for the entire 24 hours following administration of the test dose was analyzed, at definite intervals, and as soon as possible after voiding. It was necessary to know the actual quantity of ascorbic acid of the test dose excreted in the 24 hours after administration in order to determine the quantity metabolized by the body during this period.

After determining the quantity of ascorbic acid excreted in the 24 hours following administration of this test dose, it was possible to determine the quantity retained by the particular individual being studied. To do this, the quantity of ascorbic acid present in the diet was taken into account, and the level of ascorbic acid in the "fasting" urine sample was taken into consideration. By subtracting the quantity of

[2] We wish to express our gratitude to Hofmann-La Roche for supplying the sterile ampules, and for the crystalline ascorbic acid used in this study.

ascorbic acid excreted by the individual in the test period from the quantity administered (both in the diet and in the test dose), a retention figure was obtained. By subtracting the quantity of ascorbic acid excreted by this individual under "fasting" conditions from this retention figure, a value was obtained which has been considered in this study the quantity of ascorbic acid metabolized (and therefore, probably required) by the subject in question.

In the course of this study, certain metabolic relationships of ascorbic acid were investigated. Hemoglobin values and the urinary output of citric acid were determined before and after the administration of the test dose of ascorbic acid. Observations were also made on the relation of the ascorbic acid requirement to the basal metabolic rate.

The methods described by the authors in a previous paper (Purinton and Schuck, '43) were used for the determination of ascorbic and citric acids.

For the determination of hemoglobin, the method of Breuer and Militizer ('38) was used, the only modification being the use of ammonium persulfate in place of potassium persulfate suggested by these workers.

The method used for determining the human requirement values for ascorbic acid was checked for validity by means of repeated tests on one person. Altogether, five determinations were completed on this individual. The determinations were made at different seasons, to offset the possibility of the influence of fresh fruits and vegetables in the past dietary history. The greatest variation noted in the different determinations for this individual was only 6.5 mg. per day. The fact that this variation was so small was interpreted to be a good indication of the applicability and validity of this method for the determination of human requirements. The plasma content of ascorbic acid for this subject under fasting conditions was at all times well above the standard normal value used in this study (0.8 mg. ascorbic acid per 100 ml. plasma).

RESULTS

Sixty-three apparently normal individuals, 52 women, aged 16 to 49 years, and 11 men, aged 20 to 25 years, were studied by means of the saturation test dose method for determining human requirements for ascorbic acid. Forty-six per cent of these had "fasting" plasma ascorbic acid levels of 0.8 mg. or more per 100 ml. of plasma. Citric acid excretion was determined for 42 of these subjects. The test dose of ascorbic acid was administered by injection to 47 subjects and by mouth to 16 subjects. The results are summarized in table 1.

TABLE 1

Ascorbic acid requirements[1] *and citric acid excretion.*

CATEGORY OF INTEREST	AGE IN YEARS AND SEX			
	15 – 20 women	20 – 25 women	20 – 25 men	25 – 50 women
No. subjects	8	29	11	15
Aver. "fasting" plasma asc. acid, mg./100 ml.	0.72	0.71	0.76	0.88
Aver. "fasting" urine asc. acid, mg./day	20.1	25.6	19.7	28.2
Asc. acid administered in test period (diet + test dose) mg.	514	514	515	514
Aver. urinary excretion in test period, mg./day	381.4	382.8	370.6	404.9
Aver. requirement, mg./day	112.5	105.6	124.7	80.9
Aver. requirement, mg./Kg.	1.95	1.86	1.71	1.50
Aver. citric acid excreted prior to test dose, gm./day	0.55	0.38	0.54	0.69
Aver. citric acid excreted after test dose, gm./day	0.80	0.66	0.90	1.24

[1] Results obtained from orally administered doses and with subjects showing very low "fasting" plasma levels accompanied by unusually high retentions were not used in computing average asc. acid requirements.

It is of interest to note that the younger women subjects showed a higher ascorbic acid requirement than the older ones. Also it was found that the average requirement figure for those with low "fasting" plasma ascorbic acid levels was higher than that for subjects with "fasting" plasma levels of 0.8 mg. or more per 100 ml. plasma.

Hemoglobin studies were made upon 19 of the subjects. It was found that 17 or 89.5% showed a definite correlation of hemoglobin in the blood with ascorbic acid content of the plasma. Only 2 individuals or 10.5% failed to show this correlation. In general, these individuals whose concentration of ascorbic acid in the fasting blood sample was above the figure used in this study as a normal fasting level (0.8 mg. per 100 ml. plasma) also had concentrations of hemoglobin above normal (15.6 gm.-Haden). Those subjects with low concentrations of ascorbic acid in the fasting sample were found to have low levels of hemoglobin. The incidence of low hemoglobin content of the blood was greater in the women subjects than in the men.

Of 16 subjects reporting a low basal metabolic rate, 68.8% had an ascorbic acid requirement below the average.

In table 2 are found figures on the relationship between ascorbic acid retention and citric acid excretion which appear

TABLE 2

Ascorbic acid retention and citric acid excretion.

	NO. OF SUBJECTS	TOTAL CITRIC ACID EXCRETED AFTER ADM. TEST DOSE ASC. ACID AVG. GM./DAY	INCREASE IN CITRIC ACID OUTPUT AFTER ADM. TEST DOSE ASC. ACID AVG. GM./DAY
Total	39		
Retention asc. acid below avg.[1]	22	0.93 (Range 0.41–2.30)	0.41 (Range 0.08–1.08)
Retention asc. acid above avg.	17	0.75 (Range 0.25–1.54)	0.35 (Range 0.05–0.94)

[1] The average retention figure for this group following administration of test dose of ascorbic acid was 138.8.

The differences were considered as having some meaning because they represent quantities greater than the total citric acid output of some of these subjects under fasting conditions.

to confirm the findings in animal studies reported in an earlier paper (Purinton and Schuck, '43). It was observed with our human subjects that lowered retention of ascorbic acid was accompanied by an increase in the total quantity of citric acid excreted. Conversely, high retention of ascorbic acid was accompanied by a lower excretion of citric acid than was noted when retention of ascorbic acid was low.

DISCUSSION

The daily human requirements for ascorbic acid, determined in this study, were found to be higher than most figures occurring in the literature.

Since slightly more than half of the subjects had "fasting" plasma ascorbic acid levels of less than 0.8 mg. per 100 ml. plasma, the high requirement figures may reflect in part the need for ascorbic acid for tissue saturation.

An optimal intake for the young adult appears to be in excess of 100 mg. per day. For adults between 25 and 50 years of age, less than 100 mg. per day appears to represent an optimal intake. This lowered requirement for older subjects is what might be expected since bodily requirements in general tend to decrease with maturity.

The orally administered test dose for determining human requirements for ascorbic acid by the saturation test dose method did not in this study give results comparable to the injected dose. In most cases the proportion of the oral test dose excreted during the 24 hour test period was small and retention figures averaged much higher than was the case with the injected test dose.

The findings of this study seem to indicate that requirements for ascorbic acid are influenced by certain factors, such as basal metabolic rate, and hemoglobin levels.

The relationship noted between ascorbic acid retention and citric acid excretion might be interpreted as signifying that citric acid plays a definite role in the metabolism of ascorbic acid, or that ascorbic acid functions in the metabolism of citric acid.

SUMMARY

1. The daily requirements for ascorbic acid of a group of 63 individuals, 52 women and 11 men, were determined by use of the saturation test dose method administered intravenously.

2. A requirement greater than 100 mg. daily was obtained for the subjects under 25 years of age. The requirement for a small number of older women between 25 and 50 years of age was well below 100 mg.

3. A correlation between the ascorbic acid of the plasma and the hemoglobin level was found in 17 out of 19 individuals.

4. The basal metabolic rate also appeared to be related to the ascorbic acid requirement, this requirement being less for subjects with low basal metabolism.

5. Confirmation of earlier findings with animal studies on the existence of a relation between ascorbic acid retention and citric acid excretion was obtained.

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THE NUTRITIVE VALUE OF PROTEIN

I. THE EFFECT OF PROCESSING ON OAT PROTEIN

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It is perhaps not well known that cereal grain is the largest single contributor of protein to the national dietary. At the White House Conference on Child Health and Protection ('32) it was reported that 36% of the protein in the national dietary was supplied by grains while only 26% was supplied by meat, 20% by dairy products and 18% by miscellaneous sources. These figures are probably not representative of the civilian dietary today, for it is evident that less animal protein is being consumed by the average civilian than in the pre-war period.[1]

Since animal proteins have long been considered superior in quality to the plant proteins the statement that "there is no need to include protein of an animal origin in the diet of man whatever the needs to be satisfied, provided that the choice of plant foods is not too much restricted" becomes significant (Cuthbertson, '40).

In view of the knowledge that cereals have such wide-spread use and when used freely with other plant proteins may supply protein for adequate human nutrition, it is pertinent to determine how best the cereal proteins can be used to replace unavailable animal protein. The efficacy with which this is done depends in part on the biological value of the cereal in the form in which it is offered for human consumption.

[1] In Jan. 1943, the estimated average per capita consumption of meat was 93.2% of the 1941 figure (Annal. Am. Acad. Political and Soc. Sci., Jan., 1943, p. 107).

Several investigators have shown that processing of cereals affects the nutritive value of the protein. Morgan ('31) has demonstrated that severe heat treatment adversely affects wheat protein. These observations have been substantiated by Murlin and associates ('38). So far as we are aware, nothing has been reported on the effect of cooking or processing on the nutritive value of oat protein. Since nutritionists (Wilder and Keyes, '42, Maynard, '42, Murlin et al., '38) have recognized the superior protein quality of oats, it is of interest to learn whether the superior quality of oat protein is carried through to the ultimate consumer. Accordingly, an investigation of the protein quality of the various oat products that have appeared on the market has been undertaken.

Oats are generally cooked in the home, but at least three and possibly four precooked oat products are on the market and each of them is prepared by a different cooking technique, so they offered an ideal source of material for this study. These products are:

(1) Drum-dried oatmeal commonly found on the market as a precooked baby food. The product used in these tests was made from oat flour batter kept at the boiling point of water for 15 minutes and then dried in thin flakes in 15 seconds on a drum-drier held at 130° C.

(2) Oven expanded oat cereal [2] made by cooking oat flour in dough form in a jacketed tube under pressure of 100 lb. for 1 to 2 minutes, forming into globules and expanding the globules in approximately 1 to 2 minutes in an oven held at 200° C.

(3) A ready-to-eat cereal made of 75% oat and 20% corn and rye flours; made according to the patent from a dough cooked to the boiling point of water, formed into small "donuts" which are dried and subjected to a pressure of 80 to 100 lbs. at a temperature of 190° C. to 232° C. in from 5½ to 6½ minutes and exploded or puffed by releasing pressure. This product is hereinafter referred to as gun exploded oats-corn-rye cereal. [3]

[2] Quaker Oaties produced under patent No. 2,233,602.

[3] Cheerioats produced under patent No. 2,162,376.

(4) Puffed oats made by preheating oat groats for 5 minutes to a temperature of 122° C. and then subjecting the groats to live steam up to 200 lbs. 198° C. in 2 minutes and finally puffing by releasing the pressure suddenly. This latter product has been sold in limited amounts here and in Canada.

EXPERIMENTAL

The method of measuring protein quality used in these experiments is a modification of the original rat growth method of Osborne, Mendel and Ferry ('19) in which the gain in body weight per gram of protein consumed is used as an index of the protein quality of the material under examination.

Animal test groups were made up of three male and three female rats from 50 to 60 gm. in weight. Each test animal was kept in an individual screen bottom cage in an air conditioned room.

The test materials were incorporated into a basal ration in amounts that would bring the total protein ($N \times 6.25$) to 10% of the total ration. Since the basal ration had 0.5% protein, the test material supplied 9.5% protein to the ration. The other ingredients included 4% salt mixture (Hawk and Oser, '31), 2% cod liver oil, 1.5% vitamin B complex powder [4] and the balance carbohydrates. The vitamin B complex powder furnished the following vitamins per 100 gm. of basal ration: 0.9 mg. of B_1, 0.27–0.32 mg. of B_2, 0.26 mg. of B_6, 1.1–1.4 mg. of pantothenic acid. Rations 22 to 25, inclusive, were supplemented with synthetic vitamins to contain the following levels of vitamins in 100 gm. of ration: 0.9 mg. of B_1, 1.6 mg. of B_2, 1.2 mg. of B_6, 1.2 mg. of pantothenic acid, and 2.5 mg. of alpha-tocopherol.

A summary of the performance of the individual animals at the termination of the 56-day test period is given in table 1, part A. In tabulating, the data have been placed in three groups in each of which different methods of feeding were used. In the first group rolled oats,[5] toasted oat cereal

[4] Yeast concentrate No. 200 QGS Vico Products Company.

[5] Quaker Oats.

TABLE 1

Performance of individual rats on 10% protein rations at the end of a 56-day test period.

RAT	SEX	INITIAL BODY WEIGHT	GAIN IN BODY WEIGHT	RATION EATEN	AVERAGE DAILY INTAKE	GAIN PER GRAM OF PROTEIN	RELATIVE PROTEIN QUALITY
		gm.	gm.	gm.	gm.	gm.	
		A All groups fed ad libitum.					
	Group 9	*Rolled oats ration (10% protein, 5.4% fat)*					
1	male	59	105	585	10.4	1.80	
2	male	56	96	616	11.0	1.57	
3	male	59	118	692	12.4	1.71	
4	female	55	87	652	11.6	1.56	
5	female	58	94	610	10.9	1.55	
6	female	57	79	759	13.6	1.05	
Average		57	97	637	11.4	1.52	100
	Group 12	*Oven expanded cereal ration (9.5% protein, 3.4% fat)*					
1	male	59	79	500	8.9	1.67	
2	male	55	96	572	10.2	1.77	
3	male	58	95	577	10.3	1.74	
4	female	57	67	486	8.7	1.46	
5	female	57	73	558	10.0	1.39	
6	female	58	72	506	9.0	1.51	
Average		57	80	533	9.5	1.60	105
	Group 15	*Drum dried oatmeal ration (10.1% protein, 4.1% fat)*					
1	male	56	78	516	9.2	1.50	
2	male	55	71	463	8.3	1.52	
3	male	55	81	503	9.0	1.59	
4	female	61	83	535	9.6	1.54	
5	female	60	108	699	12.3	1.53	
6	female	52	76	462	8.3	1.63	
Average		57	82	530	9.5	1.55	102
		B Group 13 fed ad libitum. Daily intakes of groups 14 and 10 restricted to that of group 13					
	Group 13	*Gun exploded oats-corn-rye cereal ration (9.7% protein, 3.7% fat)*					
1	male	56	13	324	5.8	0.42	
2	male	58	11	327	5.8	0.35	
3	male	57	20	484	8.6	0.43	
4	female	55	18	357	6.4	0.52	
5	female	58	20	438	7.8	0.47	
6	female	59	16	393	7.0	0.42	
Average		57	17	387	6.9	0.44	39
	Group 14	*Rolled oats ration (9.8% protein, 5.4% fat)*					
1	male	61	40	388	6.9	1.05	
2	male	63	42	388	6.9	1.10	
3	male	57	51	388	6.9	1.34	
4	female	54	41	388	6.9	1.08	
5	female	62	39	388	6.9	1.03	
6	female	60	47	388	6.9	1.24	
Average		60	43	388	6.9	1.14	100
	Group 10	*Home cooked oatmeal ration (10% protein, 5.4% fat)*					
1	male	57	46	388	6.9	1.19	
2	male	55	49	388	6.9	1.26	
3	male	55	48	388	6.9	1.24	
4	female	54	47	388	6.9	1.21	
5	female	57	41	388	6.9	1.06	
6	female	58	45	388	6.9	1.16	
Average		56	46	388	6.9	1.19	104

and drum dried oatmeal were tested. The animals were fed ad libitum and a record of the food intake and weight changes was kept.

Although the average protein efficiency (gain/gram protein) of these three rations is practically constant there is considerable variation between individual rats within a group which may be attributed to the differences in food intake. In order to eliminate this variable in the succeeding protein quality tests the food consumption was equalized between animals and groups by limiting the amount of food given each animal to the average daily consumption of the group eating the least amount of food.

In the second experiment a ration containing gun-exploded oats-corn-rye cereal was fed ad libitum. In preliminary experiments it was found that animals on this ration tended to consume small amounts of food whereas on rolled oats their voluntary consumption was much greater. Therefore, the animals on the rolled oats and "home" cooked oatmeal rations were given a daily allowance equivalent to the average consumption of the gun-exploded cereal group on the preceding day. A summary of the performance of these animals is given in table 1, part B.

In this experiment the animals within each group show less biological variation than the animals on the unrestricted ration. It will be noted, however, that there is quite a drop in the protein efficiency of the rolled oats in the second experiment. This drop is due in part to the greatly lowered food intake in the second experiment.

It should also be pointed out that the animals on the gun exploded oats-corn-rye cereal ration tended to eat less food as the test period proceeded. This worked to the definite disadvantage of animals on other rations that showed fairly good growth responses at the beginning of the test period, and were then limited to smaller amounts of food even though their maintenance requirements had become greater. Partial starvation resulted and the protein efficiency, when calculated

over the entire feeding period, was lower than would be expected if a more balanced feeding program had been adopted.

From previous experience it was estimated that the animals on the gun exploded oats-corn-rye cereal ration would consume the least amount of food in the final series of tests and that their average daily consumption for the 56-day period would be approximately 7.0 grams. Accordingly all the animals in each group were offered 7.0 grams of their test rations each day during the test period.

The four materials tested were: rolled oats, exploded oats-corn-rye cereal, puffed oats and a 7.5 to 1 to 1 mixture of rolled oats, corn and rye flour respectively. As nearly as can be determined this latter mixture has the same cereal ingredients as the exploded oats-corn-rye cereal but has not been heat processed. In this test, the fat levels of all the rations as determined by the acid hydrolysis method were adjusted to approximately 11% by the addition of lard. Vitamins B_2, B_6 and alpha-tocopherol were also added to eliminate the possibility of a deficiency from these dietary essentials.

The results of this experiment given in table 2 confirm those of the previous test and show that the nutritive value of oat protein is definitely impaired, presumably by the severe heat treatment encountered in the explosion method.

Although three different methods of feeding have been used in these tests, it is possible to evaluate the various methods of processing since a common factor, rolled oats, appears in all three experiments. Arbitrarily assigning the value 100 to rolled oats in each of the three experiments the relative values of the other oat products can be calculated from the gain per gram of protein consumed. The values are given in the final column of tables 1 and 2.

If these values are a true indication of the relative protein quality, it can be concluded that normal cooking does not impair protein quality but may actually slightly improve the cereal. On the other hand severe heat treatment, such as is encountered in the explosion process destroys the quality of oat protein to a large extent.

TABLE 2

Performance of individual rats on 10% protein rations.
All animals offered 7.0 gm. of test ration each day of 56-day period.

RAT	SEX	INITIAL BODY WEIGHT	GAIN IN BODY WEIGHT	RATION EATEN	AVERAGE DAILY INTAKE	GAIN PER GRAM OF PROTEIN	RELATIVE PROTEIN QUALITY
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	
		Group 22 Rolled oats ration (10% protein)					
1	male	57	58	392	7.0	1.48	
2	male	54	65	392	7.0	1.66	
3	male	58	62	392	7.0	1.58	
4	female	54	59	392	7.0	1.50	
5	female	54	62	392	7.0	1.58	
6	female	60	51	392	7.0	1.30	
Average		57	60	392	7.0	1.52	100
	Group 23	*Untreated oats-corn-rye ration (10.2% protein)*					
1	male	55	53	392	7.0	1.42	
2	male	58	54	392	7.0	1.45	
3	male	54	53	392	7.0	1.42	
4	female	55	55	392	7.0	1.48	
5	female	57	58	392	7.0	1.56	
6	female	55	59	392	7.0	1.58	
Average		56	55	392	7.0	1.48	97
	Group 24	*Gun exploded oats-corn-rye cereal ration (9.5% protein)*					
1	male	57	18	366	6.5	0.48	
2	male	55	20	386	6.9	0.51	
3	male	58	18	362	6.5	0.49	
4	female	56	17	387	6.9	0.43	
5	female	57	13	362	6.5	0.35	
6	female	56	24	391	7.0	0.60	
Average		57	18	376	6.7	0.48	32
	Group 25	*Puffed oats ration (9.8% protein)*					
1	male	57	15	390	7.0	0.39	
2	male	52	11	354	6.3	0.32	
3	male	53	13	322	5.7	0.41	
4	female	52	10	349	6.2	0.29	
5	female	55	11	374	6.7	0.30	
6	female	54	11	338	6.0	0.33	
Average		54	12	354	6.3	0.34	23

SUMMARY

Several oat products processed by different commercial procedures, as well as home cooked oatmeal, have been tested for their relative protein quality by a rat growth method. The protein efficiency calculated from the gain in body weight

per gram of protein consumed indicates ordinary household preparation of oatmeal, precooking of oat flour by the drum dried process and toasting of an extruded oat flour product does not cause any impairment of the protein quality. Processing by means of the explosion technique does cause considerable damage to the protein quality of oat products.

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DETERMINATION AND CONTENT OF CAROTENE AND VITAMIN A IN WISCONSIN BUTTER[1]

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ONE FIGURE

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Most methods for determining carotene and vitamin A in butter and milk involve the use of a spectrophotometer (Baumann and Steenbock, '33; Semb, Baumann, and Steenbock, '34; Olson, Hegsted, and Peterson, '39). Willstaedt and With, '38, developed a method based on the reaction of vitamin A with antimony trichloride and measured the amount of color product with a step-photometer. Koehn and Sherman ('40) also utilized the antimony trichloride reaction for A but used the photoelectric colorimeter for the measurement. Carotene was also determined by means of this instrument. A modification of the Koehn and Sherman procedure was adopted by the Technical Comittee, and this method, with some changes in details, is that employed in this paper.

[1] In the latter part of 1941 the Committee on Food and Nutrition of the National Research Council proposed that the state experiment stations should develop a standard method for determination of vitamin A and carotene in butter and should collect samples of butters produced in the United States and analyze them for carotene and vitamin A content. Director C. H. Bailey of the Minnesota Agricultural Experiment Station, the coordinator of the project, appointed a Technical Committee consisting of L. A. Maynard (Chairman), C. A. Cary, H. R. Guilbert, I. L. Hathaway, C. J. Koehn, L. S. Palmer, W. H. Peterson, and F. P. Zscheile, to initiate the work. Copies of the method adopted by the Technical Committee may be obtained from Dr. C. A. Cary, Bureau of Dairy Industry, U.S.D.A., Washington, D. C. The data reported in this paper represent some of the results obtained at the Wisconsin Agricultural Experiment Station on these two problems. They are published with the approval of the Director of the Wisconsin Agricultural Experiment Station. The work was supported in part by a grant from the Nutrition Foundation, Inc., New York.

STANDARD CURVES FOR CAROTENE AND VITAMIN A

Beta-carotene [2] (M. P. 180° C.) was used for the construction of a standard curve with galvanometer readings given by passing the light through a 440 mμ. filter. Ten dilutions of carotene, ranging from 0.2 to 4 μg./cc. were made and read. With galvanometer readings (G_{440}) above 46 [3] the curve is represented by the factor 2.93. In this range $L_{440} \times 2.93 =$ μg. carotene per cc. L values corresponding to galvanometer readings ($L = 2 - \log G$) are supplied with the colorimeter. Below 46[3] the curve is no longer straight and the factor increases with concentration of carotene.

The standard curve for vitamin A was made with crystalline vitamin A alcohol.[3] It was stated to have an extinction coefficient of 1720. Eight dilutions of the alcohol ranging from approximately 1 μg. to 8 μg./cc. were treated with the Carr-Price reagent, the resultant color read through a 660-mμ. filter, and the readings used in the construction of the curve. The factor relating weight of vitamin A to the corresponding L value was $1.33 \pm .06$. Hence $L_{620} \times 1.33 =$ μg. vitamin A/cc.

CHROMATOGRAPHIC SEPARATION OF PIGMENTS

The naturally-occurring non-carotene pigments of butter as well as butter colors can be separated from the carotene by chromatography. The results thus obtained were used as the standard in developing the solvent-separation method. Heavy U.S.P. magnesium oxide, tested for its retention of β-carotene, was dry-packed in a tube to form a column 4 to 5 cm. long and 1.25 cm. in diameter. The solution of pigments in Skelly Solve B (hereafter called S.S.B.) was concentrated under reduced pressure to about 5 cc. and forced into the column with compressed air. The column was washed with S.S.B. until the solvent came through colorless and the fraction read as carotene. The non-carotene pigments were then eluted from the column with S.S.B. containing 10% absolute alcohol. This solution was evaporated to dryness under reduced pressure, the residue was taken up in S.S.B., and the solution read for non-carotene pigments expressed as carotene.

[2] Obtained from the S.M.A. Corporation, Cleveland, Ohio.

[3] Obtained from Distillation Products Company, Rochester, N. Y.

Diacetone alcohol diluted with a small quantity of water to 94% strength was found to remove more non-carotene pigment from S.S.B. solutions than does 92% methanol or 85% ethanol. The values obtained by the diactetone extraction procedure agreed closely with the chromatographic results.

TABLE 1

Carotene determination of colored and uncolored butters by different methods.

SAMPLE	METHOD	TOTAL PIGMENT AS CAROTENE	CAROTENE	NON-CAROTENE	RECOVERY OF TOTAL PIGMENT	CAROTENE
		μg./gm. fat	*μg./gm. fat*	*μg./gm. fat*	*%*	*% of total*
1a. No added color	Chromatograph	2.65	1.66	.94	98.2	62.7
1b. No added color	94% Diacetone extraction	...	1.64	...	...	61.8
1c. No added color	92% Methanol extraction	...	1.96	...	...	74.0
1d. No added color	85% Ethanol extraction	...	2.10	...	...	79.1
2a. No added color	Chromatograph	9.73	7.48	1.76	95	77
2b. No added color	94% Diacetone extraction	...	7.23	...	...	74.4
2c. No added color	92% Methanol extraction	...	7.72	...	...	79.5
3a. No added color	Chromatograph	9.03	7.0	1.7	96.3	77.5
3b. No added color	94% Diacetone extraction	...	6.87	...	...	76.3
3c. Added color	94% Diacetone extraction	13.35	6.83	...	...	75.7

The determinations were run on S.S.B. solutions of the butter extract obtained as described later in the paper. An aliquot was read for total pigment. A second aliquot of 20 cc. was extracted with three 10-cc. portions of diacetone, washed once with 10 cc. of water, made up to volume and read. A third aliquot was chromatographed, and a fourth was extracted with three 10-cc. portions of 92% methanol, washed three times with 10-cc. portions of water, and dried over anhydrous sodium sulfate before bringing up to volume and reading. Table 1 shows typical results obtained with both colored and uncolored butters. For all samples the diacetone figures ranged from 95 to 103% of the chromatographic values and the methanol results varied from 98 to 118%. When the carotene content was exceptionally low, as in winter butters, figures as high as 118% were obtained with methanol. These high figures are probably due to the presence of acid-formed pigments of silage. These pigments pass into the milk and appear in the butter (Peterson, Bird, and Beeson, '37; Quack-

enbush, Steenbock, and Peterson, '38). Diacetone extracts these pigments while methanol does not.

Tests were made to determine the number of washings required to remove diacetone and methanol from the S.S.B. solution of carotene. One washing proved sufficient to remove the cloudiness caused by these solvents. Three to six washings had no effect on the quantity of carotene found.

Drying the S.S.B. solution of carotene over anhydrous sodium sulfate before reading appears not only to be unnecessary but may yield erroneous results. In no case were the readings of the dried carotene solutions higher, and on several butter samples they were lower than the values for undried and chromatographed samples. These losses were later traced to the kind of Na_2SO_4 used. One, a granular product, absorbed no carotene but a second, which was very finely powdered, removed from 8 to 22% of the total carotene from solution. No such absorption of carotene or vitamin A from ether solution by anhydrous sodium sulfate was encountered.

COMPOSITION OF PIGMENTS IN THE SOLVENT FRACTIONS

The composition of the fractions obtained by extracting the S.S.B. solution of pigments with diacetone and methanol was determined by chromatographing. Before chromatographing, the extracted S.S.B. solution was washed with water to remove the interfering methanol and diacetone. The pigments in the methanol and diacetone extracts were transferred to S.S.B. for chromatographing by diluting the extracts with an equal volume of water and shaking with three 10-cc. portions of S.S.B. Table 2 contains the results of a typical analysis.

The data show that approximately 10% of the total carotene (0.64 μg. out of 6.5 μg.) was removed by the diacetone-alcohol but that non-carotene pigment (0.38 μg.) was left behind to compensate largely for this removal. Extraction with 92% methanol removed only 0.1 μg. of carotene but left behind about 0.95 μg. of non-carotene pigments. The carotene

figure by the diacetone method (6.33) checks reasonably well with the chromatographic figure (6.5), but the methanol value (7.22) is 11% too high.

TABLE 2

Chromatographic analysis of diacetone and methanol fractions.

NO.	FRACTION	TOTAL PIGMENT AS CAROTENE	CAROTENE	NON-CAROTENE	RECOVERY	CAROTENE IN FRACTION
		μg./gm. fat	*μg./gm. fat*	*μg./gm. fat*	*%*	*% of total*
1.	Skelly Solve B solution of total pigments	8.65	6.5	2.12	99.7	100
2.	Residue after extraction of 1 with diacetone	6.33	5.77	0.38	97.1	88.8
3.	Diacetone extract of 1	2.32	0.64	1.66	99.1	9.9
4.	Residue after extraction of 1 with 92% methanol	7.22	6.3	0.95	100.4	96.9
5.	Methanol extract of 1	1.43	0.1	1.17	88.8	1.5

CORRECTION FACTORS FOR PRESENCE OF CAROTENE AND ADDED BUTTER COLOR OF VITAMIN A

Antimony trichloride reacts with carotene to produce a blue color with maximum absorption at 590 mμ. but with sufficient absorption at 620 mμ. to cause an error in the calculation of A if no correction is made. A correction factor was arrived at by testing six dilutions of a carotene solution ranging from approximately 1 μg. to 11 μg./cc. The correction factors ranged from 0.0081 to 0.0059 increasing as the concentration decreased. The average of the six values was 0.007. This factor is an expression of the effect on the L value at 620 mμ. when 1 μg. of carotene reacts with antimony trichloride. It is applied by multiplying the μg. of carotene present in the Evelyn tube by 0.007 and subtracting this from the observed L value.

The dyes yellow AB and OB also react with antimony trichloride to produce a pink color with some absorption at

620 mμ. The same correction factor was found applicable to them as is applied to carotene. Therefore:

L_{620} (corrected) = L_{620} (observed) − (0.007 × μg. total pigment expressed as carotene).

SOLVENT EXTRACTION METHOD FOR ANALYSIS OF BUTTER

Whole butter may be used if the fat content is known. If not, the butterfat is obtained by melting the butter on a water bath below 60° C. and filtering it through cotton. It is then allowed to solidify in the refrigerator. The cake is removed and scraped free of any adhering particles of moisture and non-fat solids. Ten gm. of fat are weighed into a suitable saponification flask. Five gm. of solid KOH, 5 cc. of water and 20 cc. of aldehyde-free methanol are added, and the flask is connected by a ground glass joint to a condenser and refluxed on a water bath for 10 minutes. The sample is then diluted with 40 cc. of distilled water, transferred to a separatory funnel, and the flask rinsed with an additional 40 cc. of water. The mixture is extracted with one 100-cc. and two 50-cc. portions of peroxide-free ether. The combined extracts are washed with 100-cc. portions of distilled water until the washings are free of alkali. Four washings should be sufficient. The ether solution is dried over anhydrous sodium sulfate and transferred to a round-bottom Claisen flask; the sodium sulfate is washed three times with 10-cc. portions of ether and the washings added to the main solution. Several glass beads are added to prevent bumping. The flask is placed on a warm water bath and the ether removed under reduced pressure. Care must be taken not to continue heating the flask after the ether has been removed. About 15 cc. of S.S.B. is added before the vacuum is released. If the flask is still warm, it is cooled under the water tap. The solution and rinsings of the flask with S.S.B. are transferred to a 50-cc. volumetric flask and made up to volume.

A 5-cc. aliquot is diluted to 10 cc. and read for total pigment. Another aliquot of 25 cc. is extracted with three 10-cc. portions of 94% diacetone-alcohol to remove non-carotene

pigments, washed once with 10 cc. of water, made up to 50 cc. with S.S.B. and read for carotene.

For the vitamin A determination 1 cc. of the original S.S.B. solution is placed in an Evelyn tube, the S.S.B. is removed under vacuum and replaced by 1 cc. of chloroform. Nine cubic centimeters of a 20% solution of antimony trichloride in chloroform are added from a pipette with a delivery time of about 2 seconds. The galvanometer is read at the point of temporary stability.

The volumes given for carotene and vitamin A are suitable for summer butters, but for winter butters, which have less pigment, a greater concentration of the final solution may be required.

The instrument used is the Evelyn Photoelectric Colorimeter with a 440-mμ. filter for carotene and a 620-mμ. filter for vitamin A. Concentrations are read off standard curves and the proper correction factors applied.

The above method differs from that adopted by the Technical Committee in certain respects, the most important being the following:

In the Committee method the extracting solvent for removal of non-carotene pigments is 92% methanol except for winter butters produced by cows fed acid or molasses silages. For such butters 94% diacetone is recommended. Since in most cases the kind of ration is not known, 94% diacetone has been used for all samples.

In the Committee method the S.S.B. solution used for the carotene determination is washed four times with water and dried over sodium sulfate before being diluted to a suitable volume. In our method one washing and no drying is used. Our data on the effect of washing and drying did not reach the Committee in time for consideration before the formulation of the method.

CAROTENE AND VITAMIN A CONTENT OF WISCONSIN BUTTERS

Early in April, 1942, twenty-two 1-lb. samples of butter made during the week of March 23, 1942, by creameries in

southwestern Wisconsin were analyzed. In July, 1942, twenty samples of butter (fifteen from the same creameries, five from others in the same region) were analyzed. Table 3 summarizes these analyses.

TABLE 3

Carotene and vitamin A content of Wisconsin butters

	SERIES I		SERIES II	
	March, 1942	July, 1942	Sept., 1942	Jan., 1943
Number of samples	22	20	70	65
Range, μg./gm. butterfat				
Total pigment	.....	7.2 — 13.6	6.2 — 14.8	.....
Carotene	1.9 — 2.7	5.4 — 9.6	4.8 — 11.0	2.0 — 4.9
Vitamin A	4.6 — 6.8	7.4 — 10.5	5.9 — 11.3	4.2 — 7.2
Mean, μg./gm. butterfat				
Total pigment	.....	10.33	10.53	.....
Carotene	2.16 ± .04[1]	7.43 ± .26	7.66 ± .16	2.97 ± .06
Vitamin A	5.61 ± .14	8.96 ± .19	9.06 ± .14	5.89 ± .07
International units per lb. of butter	9,568	17,738	18,032	10,488

[1] Standard deviation of the mean.

With the aid of the Division of Marketing of the State Department of Agriculture, collection and analysis of Wisconsin butters was undertaken on a much larger scale in September, 1942, and January, 1943. The state was divided into nine districts, and from each the number of samples corresponding to the district's proportion of the state production were collected and analyzed. Inspectors of the Wisconsin Department of Agriculture collected these samples from the creameries, packed them in ice or dry ice in most cases,[4] and sent them directly to the laboratory together with data sheets regarding the samples. The data sheets asked for as much information (e.g., data on churning, breed of cattle, rations, etc.) as was thought pertinent in interpreting the results.

Samples from seventy different creameries were collected in September, 1942, and other samples from sixty-five of these

[4] The packing was probably not necessary. Several samples kept at room temperature for 3 days, which is longer than a sample would be in transit, showed no change in carotene or vitamin A content.

creameries were collected in January, 1943. The analyses of these samples are also summarized in table 3. The total pigment values are not given for the winter butters since these are usually artificially colored and therefore the figures would be of little significance.

In the calculation of International Units 0.6 μg. of β-carotene and 0.25 μg. of vitamin A were each taken as equal to 1 I.U. (Baxter and Robeson, '42). The average butterfat content of the butter was assumed to be 81%.

The table shows the carotene content of July butters as almost 3.5 times that of March butters, and the vitamin A content about 1.6 times as great. September values do not vary greatly from those of July. The January butters, however, show a slightly higher content of vitamin A and a considerably higher carotene content than the March samples. From September to January the carotene content decreased about 60%, while the vitamin A decreased about 35%. About 75% of the total pigment of the summer butters was carotene. The few samples of uncolored winter butters which were obtained contained 60 to 65% of the total pigment as carotene.

The distribution of the September and January samples according to their carotene and vitamin A content is shown in figure 1. The September samples show an approximately normal type of distribution, but the January samples show a much narrower spread. This concentration effect is probably related to the storage of carotene and vitamin A in the tissues of cattle and to the low content of carotene in winter rations. During the winter feeding, cattle draw on these stores to hold up the carotene and vitamin A content of the milk. Only after several months does the milk reach a constant low level. The drop in vitamin A is less than that for carotene, because the latter is constantly being converted into the former and hence acts as a reservoir for it.

The distribution of the March samples (not shown in the figure) was of the same type as in January. However, there was a further general decline with the majority of the samples

falling in the next lower divisions as compared with the January distribution.

Variations among samples at any time may be related to several factors, but breed and carotene content of the ration are probably the two most important. Samples high in carotene and low in vitamin A invariably came from districts where 70% or more of the cattle were reported to be Guernseys. In districts where Holsteins predominated, the opposite relationship existed. About ten samples for each group were obtained from districts with this preponderance of one or

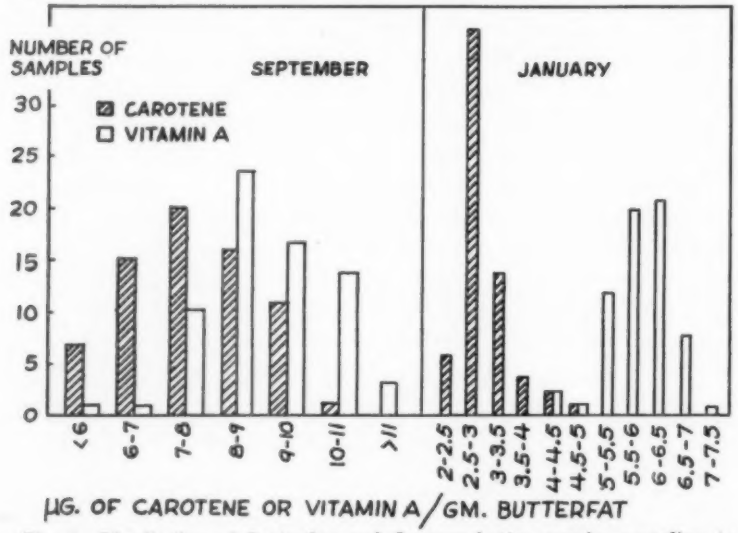

Fig. 1 Distribution of September and January butter samples according to their carotene and vitamin A content.

the other breeds. In the September series micrograms of carotene and vitamin A per gram of butterfat averaged as follows: Guernsey 9.47 and 7.34; Holstein 5.66 and 10.39. Corresponding figures for the January series were: 3.85, 5.48; 2.51, 6.50. These results bear out previous reports by different investigators on the effect of breed.

The butters were scored by Dr. L. C. Thomsen of the Department of Dairy Industry, but no correlation was found between the vitamin content and the score assigned.

In 1938 and 1939 this laboratory made a survey of the carotene and vitamin A content of market milks of the state (Dornbush, Peterson, Olson, '40). Calculating on the basis of butterfat content and correcting for the inclusion of non-carotene pigments in the milk survey values, it appears that the milks of that period were about 18% higher in vitamin A activity than the present butters. However, since the milks showed a variation of about 15% for corresponding seasons in the 2 years, this difference is not significant.

EFFECT OF STORAGE OF BUTTERS ON VITAMIN A CONTENT

In June, 1942, four samples of butter produced by the Department of Dairy Industry were analyzed prior to being placed in storage at — 21 to —23° C. These butters were stored in conventional 64-lb. unparaffined tubs lined with parchment paper and closed with wooden lids. In February they were taken out of storage and again analyzed. No loss of carotene or vitamin A was observed. The seventy 1-lb. samples obtained in September, left in the original paper wrapping and carton, were also placed in storage. Ten of these were analyzed in February, 1943. Again no loss of either carotene or vitamin A was observed.

SUMMARY

A solvent extraction method is described for the determination of carotene and vitamin A in butters, utilizing the yellow color of the carotene for its determination and the Carr-Price reaction for the determination of vitamin A.

Diacetone alcohol, 94%, proved to be superior to 92% methanol for the extraction of non-carotene pigments.

The March butters averaged about 9,500 I.U./lb. The butters of July and September averaged about 18,000 I.U./lb. The January butters were slightly better than the March butters and contained about 10,500 I.U./lb.

In the summer butters, about 75% of the total natural butter pigment was found to be carotene, in the winter butters 60 to 65% of the pigment was carotene.

Storage of butter as long as 8 months did not result in a loss of carotene or of vitamin A.

ACKNOWLEDGMENT

We wish to express our sincere thanks to Prof. L. C. Thomsen of the Dairy Industry Department for collecting the March and June samples and for supplying the samples of fresh and stored tub butters. We are greatly indebted to Mr. Milton H. Button and Mr. A. T. Bruhn of the Division of Marketing of the Wisconsin State Department of Agriculture for their fine cooperation in planning and carrying out the program of collection, and to the many inspectors of the Department of Agriculture who collected and shipped the September and January samples to the laboratory. We particularly appreciate the assistance Dr. F. W. Quackenbush has given us in setting up the standard curves for carotene and vitamin A and for counsel on other aspects of the analyses.

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THE ASCORBIC ACID REQUIREMENTS OF SCHOOL-AGE GIRLS [1]

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ONE FIGURE

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During a recent investigation in this laboratory (Roberts and Roberts, '42) concerning the vitamin C requirements of children, five subjects were tested on intakes ranging from 55 to 135 mg. On the basis of blood and urinary excretion studies, it was found that 65 to 75 mg. of ascorbic acid was needed to insure saturation of these children. It seemed pertinent to extend the investigation to include a larger number of subjects in order to determine whether the majority of children in a larger group would respond in the same way.

It is well known that many children rarely get as much as 65 mg. of ascorbic acid in the diet. The subjects in this study were tested, therefore, on lower levels of intake than had been used in the earlier study.

EXPERIMENTAL

Selection of subjects. This investigation was carried on at a suburban home for girls. Thirty pre-adolescent girls between the ages of 6 and 12 years, living in two cottages, were selected as subjects. These children were in a healthy

[1] This research was aided by the Talcott fellowship granted by Rockford College and the Omicron Nu fellowship awarded by the American Home Economics Association to the senior author.

condition although, as in most groups of children, some were underweight, and some overweight. This deviation from average weight for height and age was taken into consideration, however, in the grouping of the children.

General procedure. The subjects were divided into six groups as based upon original ascorbic acid levels of the blood, age, height, weight, and deviation from average weight. The children from each group were given daily supplements of crystalline ascorbic acid [2] for three 7-day periods. The supplements ranged from 10 mg. for the first group to 60 mg. for the sixth group.

The amount of reduced ascorbic acid in the blood plasma was determined each week. At the end of the third period, the urinary "resting level" of ascorbic acid and the response to a 300-mg. test dose were measured. The blood concentration and the response to the test dose were the criteria used in judging the state of saturation of the subjects.

Control of the diet. Determination of the dietary intake for six of the subjects over a weekly period showed that the children were getting between 31 and 94 mg. of ascorbic acid with an average of 59 mg. On this intake their blood levels ranged from 0.46 to 0.97 mg. per 100 cc.

Because of the generous amount of vitamin C-rich foods served in the institution on some days, it was necessary to make a few dietary adjustments. Tomatoes, fresh and canned citrus fruits, all raw vegetables except carrots, and all fresh fruits except apples were eliminated from the diet. The menus were adjusted so that milk and dried fruit were the only sources of ascorbic acid for breakfast. Substitutions were made in the supper so that it, too, contained very little vitamin C. Most of the ascorbic acid was obtained at the noon meal where two or three vegetables and sometimes canned fruit were served. The children did not have access to food between meals.

[2] We wish to express our appreciation to Hoffmann-La Roche, Inc., Nutley, N. J., for furnishing the crystalline ascorbic acid.

In order to determine the daily intake of ascorbic acid, the amount of food eaten by six of the subjects at the noon meal was weighed and samples of the food were analyzed. The milk and dried fruit from breakfast were also analyzed for vitamin C while the intake from the evening meal was estimated partly by analysis, and partly from tables of average composition. Inasmuch as the plates were served from the kitchen where the food had been divided quite evenly, and the children were required to "clean their plates," it seemed that the average obtained in this way would be fairly representative of the dietary intake of all subjects.

During the last 2 days of the study when urinary excretions were being determined, the diet was strictly controlled, and all foods were weighed and analyzed. In this way the intake of foods containing any vitamin C was kept the same for each subject. The average intake during each of the three weekly periods was 22, 21, and 22 mg.

Urinary determinations. The daily urinary output was collected for the last 2 days of the investigation, one day when the children were receiving their regular dietary supplement, and the other when they were given a 300-mg. test dose. To preserve the ascorbic acid in the urine, 5 N sulphuric acid and 8-hydroxyquinoline were used as suggested by Sendroy and Miller ('39).

Chemical methods. For the food and urine analyses, the method of Bessey and King ('33) was used. A buffer was added to the acidified urine to prevent the rapid fading of the end point. For blood determinations the microtechnique with the Farmer and Abt apparatus ('36) was used. All determinations were made on fasting blood plasma. The procedure of Menaker and Guerrant ('38) was employed for the standardization of the indicator, 2,6-dichlorophenolindophenol.

RESULTS AND DISCUSSION

In the present study, two criteria have been used for interpreting the results in terms of ascorbic acid requirements.

These are the same as were used in the more detailed study of Roberts and Roberts ('42). Blood levels of 0.7 mg. per 100 cc., and an excretion of 50% of a 300-mg. test dose within 24 hours are the two indices used to indicate a satisfactory state of nutrition in respect to vitamin C. Inasmuch as the daily excretion was obtained for only 1 day ascorbic acid "retention", which was one criterion in the earlier study, has not been considered in this report.

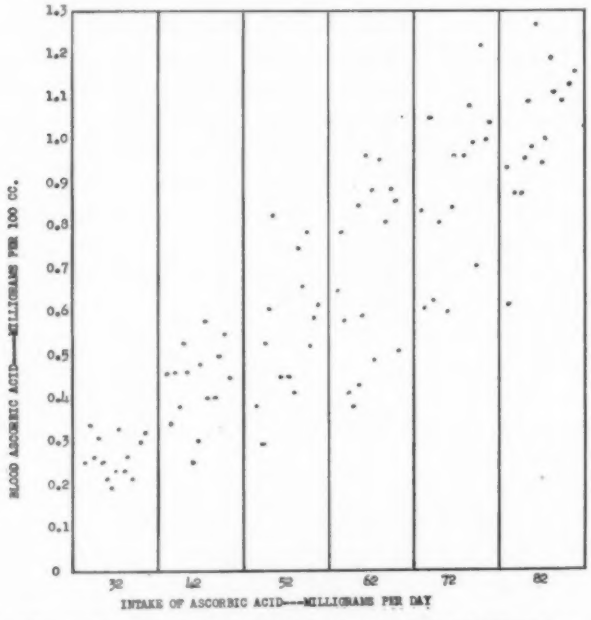

Fig. 1 The concentration of ascorbic acid in the blood plasma on various levels of intake. The figure shows results of three tests made at weekly intervals on each of thirty children.

A wide variation in blood ascorbic acid concentration on each level of intake is shown in figure 1. It will be noted that the blood levels are less variable on the lower than on the higher intakes. This variability has been observed by others. With boys living on an institution diet Roberts et al. ('39)

report blood levels ranging from 0.4 to 1.4 mg. per 100 cc. Bessey and White ('42) likewise found "considerable scattering" of blood concentrations at any one level of intake. No doubt these wide ranges are in part due to individual eating habits, and in part to variability in the response to vitamin C. Even with a controlled intake the individual blood

TABLE 1

Blood levels and excretion of ascorbic acid on various levels of intake.

GROUP	INTAKE		SUBJECT	BLOOD LEVELS MG. PER 100 CC.				EXCRETION	
				Periods			Average for 3 periods	"Resting level"	After 300 mg. test dose
	Average from food	Supplement		1	2	3			
	mg.	*mg.*						*mg.*	%
I	22	10	1	0.34	0.25	0.23	0.27	9	7
			2	0.25	0.21	0.26	0.24	7	15
			3	0.34	0.23	0.21	0.26	9	4
			4	0.26	0.19	0.30	0.25	8	22
			5	0.31	0.33	0.32	0.32	7	6
II	22	20	6	0.34	0.46	0.40	0.40	8	14
			7	0.46	0.25	0.40	0.37	10	22
			8	0.46	0.30	0.50	0.42	12	32
			9	0.38	0.48	0.55	0.47	14	39
			10	0.53	0.58	0.45	0.52	8	37
III	22	30	11	0.88	0.83	0.79	0.83	14	72
			12	0.38	0.45	0.52	0.45	10	77
			13	0.29	0.45	0.41	0.38	27	8
			14	0.53	0.75	0.59	0.62	13	90
			15	0.61	0.66	0.62	0.63	14	46
IV	22	40	16	0.65	0.85	0.96	0.82	23	78
			17	0.79	0.59	0.81	0.73	21	68
			18	0.58	0.97	0.89	0.81	28	65
			19	0.41	0.89	0.86	0.72	22	58
			20	0.38	0.49	0.51	0.46	10	32
V	22	50	21	0.84	0.60	1.05	0.83	31	71
			22	0.61	0.85	0.71	0.72	42	72
			23	1.06	0.97	1.23	1.09	34	79
			24	0.63	0.97	1.01	0.87	33	71
			25	0.81	1.09	1.05	0.98	14	75
VI	22	60	26	0.94	1.10	1.20	1.08	27	81
			27	0.62	0.94	1.12	0.89	..	..
			28	0.88	1.28	1.10	1.09	44	83
			29	0.88	0.95	1.14	0.99	22	45
			30	0.96	1.01	1.17	1.05	26	83

levels vary. With five subjects on the same diet Roberts and Roberts ('42) found differences as great as 0.3 mg. per 100 cc.

The blood levels and the urinary response of each child to a 300-mg. test dose are shown in table 1. Holmes, Cullen and Nelson ('41) have pointed out that repeated determinations of the blood ascorbic acid over a period of time constitute a more accurate measure of storage than a single determination. The blood levels during the three periods on the supplemented diet and also the mean value are included in the table. If a blood level of 0.7 mg. or above per 100 cc. is considered an indication of normal vitamin C nutrition, it is evident that the the children in groups I and II are subnormal. All of the children in these two groups were below 0.7 mg. per 100 cc. not only at the end of the first week, but during the entire 3 weeks of supplementation. Furthermore, the urinary "resting level" was low, and the response to the test dose was poor.

In the third group, only one child maintained a blood level above 0.7 mg. per 100 cc. Three in the group excreted 50% of the test dose. If the two criteria are applied as indications of vitamin C saturation, it is evident that subject 11 is the only child saturated on an intake of 52 mg.

The subjects in group IV who received 62 mg. showed a definite improvement. Though their blood levels were low during the first period, with the exception of subject 17, all blood levels increased during the second week, and these higher levels were maintained during the third period. Subject 20, however, is the only one whose mean blood level was below 0.7 mg. per 100 cc. Moreover, all except this girl excreted more than 50% of the test dose. No explanation can be offered as to the reason for this subject maintaining the low blood and excretion values. The urinary "resting level" for the other four subjects increased above the 20-mg. level, but fell short of the 40-mg. "resting level" considered by Van Eekelen and associates ('37) to be indicative of tissue saturation.

That the next two groups, V and VI, were in a good state of vitamin C nutrition is evidenced by the high blood levels, the increased excretion, and the favorable response to the test dose. All had higher blood values at the end of the third period than they had at the end of the first, and their mean blood levels were above 0.7 mg. per 100 cc. With the exception of subject 29, all in these two groups excreted more than 50% of the test dose.

Examination of the mouths of the subjects by a dentist showed that there was no relation between either the intake or the blood levels of ascorbic acid and the condition of the gums. Three weeks on a low ascorbic acid intake is evidently too short a period to expect any visible tissue changes.

The data show that under the conditions set up, 52 mg. is the lowest intake of ascorbic acid on which any of the children were saturated; only one child, however, reached saturation on this intake. Four-fifths of the subjects receiving 62 mg., and all getting 72 mg., were saturated. Thus it appears that 62 to 72 mg. would be a satisfactory level at which to set the standards for the school-age child.

The results of this study are in fair agreement with other investigations on the school-age child. As judged by excretion studies, Harris ('40) found approximately 50 mg. of ascorbic acid sufficient to saturate boys 11 to 13 years old. Pemberton ('40) reports 63 mg. for boys from 10 to 14 years old. When judged by the response to the test dose alone, it will be noted in the present study that three of the five children receiving 52 mg. of ascorbic acid were saturated. Only one of these subjects, however, had a blood level above 0.7 mg. per 100 cc. Using the two criteria, 62 mg. was found more nearly adequate.

On the basis of blood studies Bessey and White ('42) report 40 to 50 mg. daily, sufficient to maintain a high plasma concentration. The intake was estimated, however, on the consumption of citrus fruit and tomatoes only. This amount could be better compared with the supplements used in the present investigation. On this basis there is close agreement between the results of the two studies.

Furthermore, this investigation substantiates the results found in the more detailed study of Roberts and Roberts ('42), where it was found that 65 to 75 mg. were required to saturate the subjects when the same criteria as were used in the investigation were employed to judge saturation.

Moreover, the values obtained here further corroborate the allowance set up by the Committee on Food and Nutrition of the National Research Council ('41).

SUMMARY

The ascorbic acid requirements of thirty pre-adolescent girls between the ages of 6 and 12 years were studied. Blood concentrations and the urinary response to a test dose were determined on intakes ranging from 32 to 82 mg. of ascorbic acid. Blood levels of 0.7 mg. per 100 cc. and the excretion of 50% of a 300-mg. test dose in 24 hours were used as the criteria indicating a satisfactory state of nutrition in respect to vitamin C.

None of the children on intakes of 32 or 42 mg. of ascorbic acid, and only one on an intake of 52 mg. were receiving sufficient vitamin C to maintain these standards. Four-fifths of those receiving 62 mg. and all who received 72 mg. were saturated.

On the basis of this study 62 to 72 mg. of ascorbic acid would seem to be an adequate allowance for pre-adolescent girls of 6 to 12 years.

ACKNOWLEDGMENT

We wish to express our deepest appreciation to the administrators and teachers of the Park Ridge School for Girls for allowing us the privilege of working in that institution, and for their fine cooperation throughout the investigation.

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MEAD JOHNSON AND COMPANY
'B-COMPLEX' AWARD

Nominations are solicited for the 1944 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B Complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1943 must be in the hands of the Secretary by January 10, 1944. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

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SECRETARY, AMERICAN INSTITUTE OF NUTRITION